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Title: Serological and Biological Characterization of Seed-borne Isolates of Blackeye Cowpea Mosaic and Cowpea Aphid-borne Mosaic Potyviruses in *Vigna unguiculata* (L.) Walp.

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Richard O. Hampton

Five viruses were detected in cowpea plant samples from Pakistan: blackeye cowpea mosaic (BlCMV), cowpea aphid-borne mosaic (CABMV), cowpea mottle virus (CPMoV), cowpea severe mosaic virus (CSMV), and southern bean mosaic virus (SBMV). Neither cucumber mosaic virus (CMV) nor cowpea mosaic virus (CPMV) were detected in these samples. Only CABMV was found to be seed-transmitted in four of seven seed lots at rates of 0 to 7 percent. Although all viruses occurring in cowpea in Pakistan were not identified, the present study has probably determined the principal seed-borne viruses of cowpea.

Fourteen of 158 U.S. *Vigna unguiculata* Plant Introduction accessions tested for seed-borne potyviruses were found to be infected with BlCMV (6.9%) and CABMV

(13.3%) potyviruses, and seven with CMV (2.0%) cucumovirus.

Forty-three isolates of BlCMV and 55 of CABMV were derived from *Vigna unguiculata* accessions, pre-introductions and commercial cowpea plantings. Viral identities were determined by DAS-ELISA serology and verified by electron microscopy and host reactions.

The highest seed-transmission rates of 48.5% and 55% were produced by BlCMV isolate PI-25B1 and CABMV isolate RN-27C respectively. Aphid transmissibility of these viruses by *Aphis craccivora* Koch, using three aphids per inoculated plant, ranged from 24% to 55% among BlCMV isolates and 18% to 57% among isolates of CABMV.

Antiserum produced against a typical CABMV isolate RN-7C, yielded CABMV-specific immunoglobulin G, when used in DAS-ELISA.

Twenty-one seed-borne isolates each of BlCMV and CABMV were compared for pathogenicity on 51 cowpea genotypes, and found to vary markedly in both pathogenicity and virulence. Based on differential reactions of these genotypes, 21 distinct variants of BlCMV and 25 of CABMV were identified. Two genotypes, TVU 401 and TVU 1582, were immune to all isolates of CABMV. Two other genotypes, TVU 2657 and TVU 3433, were immune to all isolates of BlCMV except those naturally seed-transmitted in the cowpea cultivar 'Pusa Phalguni' (India).

Serological and Biological Characterization of Seed-borne Isolates of Blackeye Cowpea Mosaic and Cowpea Aphid-borne Mosaic Potyviruses in *Vigna unguiculata* (L.) Walp.

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Dedicated to my parents and brother

Khushi Muhammad.

ACRONYMS AND ABBREVIATION

Term	Description
ACP:	Antigen coated plate
BCMV:	Bean common mosaic virus
BlCMV:	Blackeye cowpea mosaic virus
BYMV:	Bean yellow mosaic virus
CABMV:	Cowpea aphid-borne mosaic virus
CCMV:	Cowpea chlorotic mottle virus
CMMV:	Cowpea mild mottle virus
CMV:	Cucumber mosaic virus
CPMV:	Cowpea mosaic virus
CSMV:	Cowpea severe mosaic virus
CYVV:	Clover yellow vein virus
CCl_4:	Carbon tetrachloride
CYMV:	Cowpea yellow mosaic virus
DSMV:	Dasheen mosaic virus
DAC:	Direct antigen coated
DAS:	Double antibody sandwich
ELISA:	Enzyme-linked immunosorbent assay
EM:	Electron microscopy
IgG:	Immunogammaglobulin
IITA:	International Institute of Tropical Agriculture
IMV:	Iris mosaic virus
LMV:	Lettuce mosaic virus
MAb:	Monoclonal antibody
NWFP:	North Western Frontier Province
PSbMV:	Pea seed-borne mosaic virus
PStV:	Peanut stripe virus
PSV:	Peanut stunt virus
PVY:	Potato virus Y
SBMV:	Souther bean mosaic virus
SbMV:	Soybean mosaic virus
SCMV:	Sugarcane mosaic virus
SDS:	Sodium dodecyle sulfate
SHMV:	Sunhemp mosaic virus
TSEV:	Tobacco severe etch virus
TSV:	Tobacco streak virus
TEV:	Tobacco etch virus
TVU:	Tropical *Vigna unguiculata*
WLMV:	White lupin mosaic virus
WMV:	Watermelon mosaic virus

SUMMARY

Analyses of 151 cowpea samples from Punjab and North Western Frontier Province (NWFP) of Pakistan, indicated the occurrence of five cowpea viruses: BlCMV (8%), CABMV (29%), CPMoV (3.3%), CSMV (16.5%) and SBMV (21.2%). Neither CMV nor CPMV were detected in these samples.

Seven seed lots of cowpea from Pakistan were also evaluated for the detection and identification of seed-borne potyviruses. Surprisingly, only CABMV was found to be seed-transmitted in these seed lots, at rates of 0 to 7 percent. Although all viruses occurring in cowpea in Pakistan were not identified, the present study has determined the principal seed-borne viruses of cowpea, and this is believed to be the first record of these viruses in Pakistan.

A sample of 158 *Vigna unguiculata* Plant Introductions and germplasm accessions was tested for seed-borne potyviruses. Of these, 21 accessions representing eight diverse countries of seed origin were found to be infected either with BlCMV (0.0 to 6.9%) and CABMV (0.0 to 13.3%) potyviruses, or CMV (0.0 to 2.0%) cucumovirus.

Forty-three isolates of BlCMV and 55 of CABMV were derived from *Vigna* Plant Introduction/germplasm accessions and field-grown plants. Identities of BlCMV and CABMV isolates were determined by DAS-ELISA serology and verified

by electron microscopy (EM), and host reactions. CABMV was not previously known to occur in the continental U.S.A.

Eight isolates of BlCMV and nine of CABMV were purified by three comparative potyvirus purification methods, including several host-isolate combinations. Average yields of 2.96 mg and 4.42 mg/100 g infected tissue were achieved for BlCMV and CABMV isolates respectively. Carbon tetrachloride (CCl_4) when used in combination with chloroform as clarifying agents improved purification of BlCMV, but was harmful for CABMV isolates. Borate buffer was superior over phosphate buffer for purification of both viruses.

Antiserum produced against a typical isolate of CABMV, RN-7C yielded immunoglobulin G that was effective in distinguishing between BlCMV and CABMV as well as many other potyviruses.

Twenty-one seed-borne isolates each of BlCMV and CABMV were compared for pathogenicity on 51 cowpea genotypes. Selected isolates of both viruses were also compared on 11 genotypes of bean, 7 of pea and 3 of local lesion hosts. Isolates of both viruses varied markedly in their pathogenicity and virulence. The resulting host reactions included immunity, resistance, tolerance and susceptibility. Some isolates of both viruses (PI-25B1, PU-7B, PU-8B, RN-18C, PI-39C) induced hypersensitive response in selected cowpea genotypes associated with local lesions.

Based on differential reactions of cowpea genotypes, 21 distinct variants of BlCMV and 25 of CABMV with narrow to wide ranges of pathogenicity and virulence were identified. BlCMV isolate PI-25B5, PU-7B and PU-10B and CABMV isolates CABMV-Mor, RN-18C and PI-39C expressed highest virulence. Immune and resistant sources were identified to individual BlCMV and CABMV variants. Two cowpea genotypes TVU 401 and TVU 1582 were immune to all isolates of CABMV. Two other genotypes TVU 2657 and TVU 3433 were immune to all BlCMV isolates except those (PU-7B, PU-8B and PU-10B) naturally seed-transmitted in cowpea cultivar 'Pusa Phalguni' (India).

Rates of seed-transmission were investigated for 10 isolates of BlCMV and 12 of CABMV in three or four cowpea genotypes. The highest seed-transmission rates of 48.5% and 55% were produced with BlCMV isolate PI-25B1 and CABMV isolate RN-27C respectively.

Three selected isolates each of BlCMV and CABMV were tested for non-persistent aphid (*Aphis craccivora* Koch) transmissibility using three aphids per inoculated plant. Transmission rates ranged from 24% to 55% for BlCMV and 18% to 57% for CABMV isolates.

Twenty seed-borne isolates of BlCMV and 37 of CABMV were compared by DAS-ELISA, monoclonal antibody (MAb) II-463, and by SDS-immunodiffusion tests. DAS-ELISA, monoclonal antibody, and SDS-immunodiffusion tests clearly distinguished BlCMV isolates from isolates of CABMV.

Based on DAS-ELISA, monoclonal antibody, SDS-Immunodiffusion tests and biological behavior of several seed-borne isolates of BlCMV and CABMV on selected cowpea genotypes (TVU 401, TVU 1582, TVU 2657, and TVU 3433), it was concluded that BlCMV and CABMV were two distinct potyviral entities.

Sixty-six virus-infected samples from major cowpea growing areas of Senegal (West Africa) were tested for the possible presence of seven viruses by DAS-ELISA. Tests results revealed the presence of CABMV (52%), CSMV, CPMoV, and SBMV among these samples. Two new potyvirus isolates, (V1-1 and V17-14) were seed-borne in advanced Senegal cowpea lines and were readily transmitted non-persistently by *Aphis craccivora* Koch. Both isolates were detected by use of potyvirus-selective monoclonal antibody (II-197), serologically unrelated to BlCMV and CABMV and were partially characterized. Seven cowpea genotypes, TVU 401, TVU 408P2, TVU 410, TVU 1000, TVU 1016-1, TVU 1582 and White Acre-BVR were immune to both potyvirus isolates.

Serological and Biological Characterization of Seed-borne Isolates of Blackeye Cowpea Mosaic and Cowpea Aphid-borne Mosaic Potyviruses in *Vigna unguiculata* (L.) Walp.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp. is an important grain legume crop and constitutes one of the most important sources of protein to people in many countries of the world. Cowpea is indigenous to Africa from which it was introduced into other tropical and subtropical countries (Cobley and Steele, 1975). It is now grown in many regions of Africa, India, Brazil, U.S.A., the West Indies, Australia, Pakistan and parts of Europe and South America (Rachie and Roberts, 1974). Annual worldwide production of cowpea is estimated at 2.5 million metric tons of dry beans harvested from 9 million hectares. About 20 percent of the total grown cowpea is consumed as a fresh vegetable. An estimated 80,000 hectares of cowpea are grown in the United States each year for vegetable or dry bean use (Fery, 1990). In Pakistan cowpea is planted as spring (March - June) or summer (July - October) crops. The total cowpea area in Pakistan is about 16.4 thousand hectares, with annual production of 7.8 thousand metric tons.

Viral diseases are considered to be a major yield constraint wherever cowpeas are grown, but most seriously

reduce yield in the tropical regions of the world such as Africa, Asia, and Latin America. Worldwide, more than 20 viruses have been identified as naturally infecting cowpea (Thottappilly and Rossel, 1985; Mali and Thottappilly, 1986). Numerous viruses are experimentally infectious to cowpea and should be considered potential natural threats to cowpea production (Kuhn, 1990). Cowpea viruses are endemic in the southern U.S.A., from the east coast to Texas. Viruses also affect California production areas, but appear to be of less economic importance than in the eastern states (Patel, 1985). Nine viruses: blackeye cowpea mosaic virus (BlCMV), cowpea chlorotic mottle virus (CCMV), cucumber mosaic virus (CMV), cowpea severe mosaic virus (CSMV), peanut mottle virus (PeMoV), peanut stunt virus (PSV), southern bean mosaic virus (SBMV), sun-hemp mosaic virus (SHMV), and tobacco streak virus (TSV), have been reported to naturally infect cowpea in the United States (Kuhn, 1990), and all are seed transmitted except CCMV. In Pakistan a whitefly-transmitted cowpea yellow mosaic virus is known to occur (Ahmad, 1978), but five additional viruses recently were found to naturally infect cowpeas in Pakistan (Bashir and Hampton, 1991).

More than 200 viruses are known to be seed transmitted (Mandahar, 1981). Although seed transmission

of viruses in cowpea has been known for many years, their importance increasingly has been recognized. Seed transmission plays a vital role in the ecology of crop viral diseases: carrying the virus from one season to the next, long distance transport of virus, and in providing foci of primary infection. Such epidemiological factors have caused concern for international exchange of seed-propagated crops and plant genetic resources. Notable instances of germplasm-borne viruses have been reported, e.g. pea seed-borne mosaic virus (PSbMV) in pea (*Pisum sativum*) (Hampton and Braveman, 1979), Psbmv in lentil (*Lens culinaris*) (Hampton, 1982a), bean common mosaic virus (BCMV) in beans (*Phaseolus vulgaris*) (Klein et al., 1988) and several others are considered likely to occur as seed-borne pathogens in germplasm collections of major crop plant species (Hampton et al., 1982b, Hampton, 1983). In several instances international exchanges of crop germplasm have resulted in inadvertent introduction of exotic seed-borne viruses.

More viruses are transmitted through seeds of cowpea than any other crop species (Hampton, 1983). It is therefore desirable to develop an understanding about these viruses, particularly about seed-borne potyviruses, interrelationships of which are still not well defined. There is currently free interchange of *Vigna unguiculata* germplasm, and growing evidence that seed-borne viruses

commonly accompany these exchanges. It seemed appropriate, therefore, to examine the identities and relationships of potyviruses occurring in selected U.S. *Vigna* Plant Introductions and in Pakistan cowpea crop and *Vigna unguiculata* germplasm accessions.

Among the seed-borne potyviruses reported in *Vigna unguiculata* (L.) Walp., blackeye cowpea mosaic (BlCMV) and cowpea aphid-borne mosaic (CABMV) potyviruses are economically the most important. Although BlCMV and CABMV are serologically related but are distinct potyviruses (Taiwo et al., 1982, Matthews, 1979). Recent evidence indicates that there is much confusion between the two. Isolates identified as CABMV from Kenya, Nigeria and Tanzania are now considered as BlCMV (Taiwo and Gonsalves, 1982; Taiwo et al., 1982; P.N. Patel, personal communication). Recently, Dijkstra et al. (1987) proposed dropping the name CABMV in favor of BlCMV on the basis of symptomology, serology, and host range comparisons. Previous studies conducted to demonstrate the relationships between BlCMV and CABMV were limited to only a few isolates of both the viruses; therefore, decisive relationships were precluded. From this investigation, we report serological and biological comparisons among 32 isolates of BlCMV and 50 CABMV isolates derived from selected *Vigna unguiculata* germplasm accessions, Plant Introductions, and field-grown plants to establish their relationships.

Because CABMV is assumed not to occur naturally in the U.S. (Kuhn, 1990), tests of *Vigna* germplasm from selected parts of the world seemed especially important. For example, CABMV has been reported from numerous countries (Bock and Conti, 1974) from which *Vigna* germplasm has been introduced into U.S. collections. Logic would, therefore strongly suggest that CABMV has been introduced as a seed-borne virus in *Vigna* accessions from countries where CABMV is known to occur (be indigenous, particularly East Africa and West Africa). Because the relationship between BlCMV and CABMV remains controversial, it seemed important to examine and compare both viruses as probable contaminants of the U.S. *Vigna* germplasm collections.

The specific objectives of this thesis were: (a) to survey viral diseases of Pakistan cowpea crop and to identify the potyviruses of cowpeas, (b) to identify seed-borne potyviruses in U.S *Vigna* Plant Introductions and in Pakistan *Vigna* germplasm accessions, (c) to compare a reasonable number of seed-borne isolates of BlCMV and CABMV potyviruses on the basis of their serological and biological properties to elucidate the relationship between the two viruses.

LITERATURE REVIEW

Potyviruses are among the most prevalent and important viruses in leguminous crops because they are seed-borne and are readily transmitted non-persistently by many aphid species. Among potyviruses naturally infecting cowpea crops, BlCMV and CABMV are seed-borne (Bock and Conti, 1974, Phatak, 1974, Kuhn, 1990, Purcifull and Gonsalves, 1985; Mali et al., 1988), and are considered economically most important. Literature pertaining to BlCMV and CABMV is reviewed in the following paragraphs.

A. Blackeye cowpea mosaic virus (BlCMV):

a. History, geographical distribution and major incidence:

BlCMV was first reported on cowpea in the U.S. in 1955 (Anderson, 1955). There has been some confusion on the status of BlCMV. BlCMV was assumed to be a strain of bean yellow mosaic virus (Corbett, 1956), while Uyemoto et el. (1973) reported that BlCMV and bean common mosaic (BCMV) were serologically identical. On the basis of serological, cytological and biological studies (Lima et al., 1978, 1979; Taiwo et al., 1982) BlCMV is now considered to be neither of the above, but a member of potyvirus group (Hollings and Brunt, 1981: Matthews, 1982).

BlCMV was thought earlier to have restricted geographical distribution, but it is now known to occur in

the U.S. (Anderson, 1955; Zettler and Evan, 1972, Lima et al., 1979; Murphy et al. 1987), Kenya and Nigeria (Taiwo, et al., 1982), Brazil (Lin et al, 1981), India (Mali and Kulthe, 1980a, Sekar and Sulochana, 1983, Mali, et al., 1988), Japan, (Hino, 1960), Taiwan (Chang, 1983), Thailand and Malaysia (Tsuchizaki et al.,1984), and Pakistan (Bashir and Hampton, 1991). BlCMV had also been reported in yard-long bean or asparagus bean *Vigna unguiculata ssp. sesquipedalis* in the Netherlands (Dijkstra et al., 1987).

A 25-90% incidence of naturally BlCMV-infected cowpea plants was reported by Kuhn (1990). A survey of cowpea diseases in South Carolina, conducted in 1981 and 1982 indicated an incidence of BlCMV infection ranging from 0.5 to 56.5 % (Collins et al., 1984). Mixed infection of BlCMV and CMV under greenhouse conditions caused synergistic reactions which had a devastating effect (42-85 %) on yield (Harrison and Gudauskas, 1968; Pio-Ribeiro et al., 1978). Simultaneous infection with CMV is common in the eastern states of the U.S., and results in cowpea plant stunting (Pio-Ribeiro et al., 1978) and rugose mosaic of asparagus bean (Chang, 1983).

b. Viral molecular and/or particle characteristics, purification, and relationship with other potyviruses:

BlCMV is characterized by flexuous filamentous particles with a modal length of 743 - 765 nm (Lima et al., 1979; Pio-Ribeiro et al., 1978; Taiwo et al, 1982; Mali et

al., 1988; Murphy, 1984). The coat protein constitutes 95 % of the particle weight based on A_{260}/A_{280} ratio. Coat protein subunit molecular weight is 3.4-3.5 X 10^3 (Lima, et al. 1979; Taiwo et al., 1982, Zhao et al., 1991). Nucleic acid is single stranded, positive sense RNA, with a molecular weight of 2.9 X 10^6 (Murphy, 1984). The sedimentation coefficient (S20, w) is 157- 159 S (Lima et al., 1979). RNA is infective (Taiwo et al., 1982; Murphy, 1984). The thermal inactivation point in sap from the infected cowpea plant is 60-65 C, longevity in vitro is 1-2 days, and the dilution end point is between 10^{-3}- 10^{-4} (Lima et al., 1979).

Several methods for the purification of virus particles and cylindrical inclusions of BlCMV have been described (Lima et al., 1979; Purcifull and Gonsalves, 1985; Taiwo et al., 1979, Zaho et al., 1991, Dijkstra et al., 1987) from infected tissue of cowpea or *Nicotiana benthamiana*. The yield of purified virus varies with virus isolate-host combination. The yield from systemically infected leaves of cowpea ranges from 3-8 mg per 100 g of tissue when plants are harvested 13-15 days after inoculation. Maximum yield of purified virus (20 mg per 100 g of tissue) of BlCMV-AC isolate was obtained from white lupin, when infected leaves were harvested 3 weeks after inoculation (Zaho et al., 1991).

BlCMV is considered a member of the potyvirus group

(Hollings and Brunt, 1981; Matthews, 1982). The virus is serologically related to numerous other potyviruses (Shepard et al., 1974; Lima et al., 1979), a phenomena that is common within the group. Among potyviruses, BlCMV is more closely related serologically to BCMV (Lima et al., 1979; Taiwo and Gonsalves, 1982) and peanut stripe virus (Demski et al., 1984), and shares no antigens with many other potyviruses, including soybean mosaic virus (SbMV) and bean yellow mosaic virus (Taiwo and Gonsalves, 1982). A seed-borne potyvirus of cowpea from India (Mali and Kulthe, 1980) is serologically related to BlCMV. Asparagus bean virus from Japan is also serologically related to BlCMV (Taiwo et al., 1982). Mali et al.(1988) reported serological relationship of a seed-borne isolate of BlCMV from cowpea with BYMV, BCMV, SbMV, but not with peanut mottle virus (PeMoV). Isolates previously studied as CABMV (CABMV-Kenyian & CABMV-Nigerian) (Bock, 1973) were later identified as BlCMV. Similarly, CABMV isolate from Tanzania studied by Patel and Kuwite (1982) has been recognized as BlCMV (P. N. Patel; personal communication and our observations). Dijkstra et al. (1987) compared two isolates of BlCMV (one derived from seeds of *Vigna unguiculata* ssp. *sesquipedalis* and one from *Glycine max*) with two isolates of BlCMV from U.S. (BlCMV-Fla and BlCMV-NR) and a Morocco isolate of CABMV. They concluded that all of the isolates were serologically identical and

also proposed to drop the name of CABMV in favor of BlCMV. The relationship between BlCMV and CABMV is still controversial. Direct comparison of the type isolate of BlCMV (Anderson, 1955) and the type isolate of CABMV (Lovisolo and Conti, 1966) is not possible because the original type isolates of both viruses are no longer available (Edwardson et al., 1972, Murphy et al., 1987, Purcifull and Gonsalves, 1985). The collective work of Taiwo et al.(1982) , Purcifull and Gonsalves (1985), Mali et al.(1988), and our results suggest that BlCMV and CABMV are serologically related, but are two distinct potyviruses.

c. Viral host range and virus-induced symptoms:

BlCMV has been reported to infect 40 species in 22 genera, including 34 species in 18 genera of Leguminosae family (Edwardson and Christie, 1986).

The type of symptoms of and susceptibility to BlCMV depends on the host species and cultivar and on viral strains being tested (Anderson, 1955; Kuhn, 1990). BlCMV produces both localized and systemic symptoms on cowpea. Localized symptoms include large reddish, often ring-like lesions which typically spread along the veins, forming a reddish-net pattern. Systemic symptoms include mottle, green vein banding often with interveinal chlorosis, stunting, and leaf distortion (Anderson, 1955; Thottappilly and Rossel, 1985). The virus naturally infects cowpea (*Vigna unguiculata*), asparagus bean (*Vigna unguiculata* ssp.

sesquipedalis) *Crotalaria spectabilis*, Alyce-clover (*Alysicarpus vaginalis*) and Desmodium, with cowpea being a major natural host.

d. Viral strains/variants:

Symptoms and host range variants of BlCMV have been reported (Bock, 1973; Bock and Conti, 1974, Taiwo et al. 1982, Murphy et al., 1987; Murphy, 1984). A major symptom variant is an isolate of BlCMV which causes red, necrotic ring spots and reddish veinal necrosis on cowpea cv Knuckle Purple Hall (Murphy et al., 1987). Other differences in host range between this and another isolate of similar origin to that of Lima et al.(1979), also were reported. Isolates from different parts of the world are closely related serologically (Chang, 1983; Murphy, 1984).

e. Relation of virus (and viral products) to host cells:

Cytoplasmic cylindrical inclusions (pinwheels and scrolls) have been detected in most tissues (Zettler et al. 1967, Edwardson et al., 1972; Edwardson, 1974; Lima and Purcifull, 1979, Murphy, 1984, Zhao et al., 1991) of cowpea, crotalaria, and other hosts. Nuclear inclusions have been reported in epidermal cells of *Crotalaria* infected with some isolates (Zettler et al., 1967; Edwardson, 1974, Christie and Edwardson, 1977). Based on morphology of scrolls and pinwheels in infected tissues, Edwardson assigned BlCMV to sub-division 1 of his potyvirus classification.

f. Transmission through seeds and by vectors:

BlCMV is seed-borne in cowpea. Seed transmission is dependent on both cowpea cultivars and viral isolates, and ranges from 3.5 to 55.0% (Anderson, 1955; Gay and Windstead, 1970; Zettler and Evans, 1972; Mali et al., 1983, Mali and Kulthe, 1980a; Mali et al., 1988). The highest seed transmission (71 %) of BlCMV with Pusa isolate in cowpea cv Pusa Phalguni was observed in our laboratory. Cowpea cultivars resistant to seed transmission have been reported (Zettler and Evan, 1972; Ladipo, 1977).

BlCMV is readily transmitted mechanically and in a non-persistent manner by the aphids *Aphis craccivora*, *A. gossypii*, *Macrosiphum solinifolii*, and *Myzus persicae* (Anderson, 1955; Mali and Kulthe, 1980a; Pio-Ribeiro et al., 1978; Zhao et al., 1991). Individuals of *M. persicae* were able to acquire and transmit both BlCMV and CMV from doubly-infected asparagus bean plants (Chang, 1983).

g. Priority control measures:

Resistance to BlCMV in cowpea has been reported (Kuhn et al., 1965; Lima et al., 1979; Mali et al., 1988; Walker and Chambliss, 1981; Strniste, 1987, Kuhn et al., 1966, Collin et al., 1985; Bashir and Hampton, 1992) and has been found to be controlled either by a single recessive gene (Walker and Chambliss, 1981; Taiwo et al., 1981) or a single dominant gene (Strniste, 1987, Quattara and Chambliss, 1991).

B. Cowpea aphid-borne mosaic virus (CABMV):

a. History, geographical distribution and major incidence:

CABMV has a wider geographical distribution than BlCMV. The virus was first reported from Italy in cowpea (Lovisolo and Conti, 1966). The virus was subsequently reported to occur in cowpea in Kenya (Bock, 1973), Nigeria (Ladipo, 1976; Rossel, 1977), Morocco (Fischer and Lockhart, 1976), Tanzania (Patel and Kuwite, 1982), Uganda (COPR, 1981), Zambia (COPR, 1981), Iran (Kaiser and Mossahebi, 1975), Japan (Tsuchizaki et al., 1970b), India (Mali et al., 1981), Pakistan (Bashir and Hampton, 1991), the Philippines (Beningo and Favali-Hedyat, 1977), Australia (Behncken and Maleevsky, 1977), Brazil (Lima et al., 1981; Lin et al., 1981), Iraq (Felga et al., 1981), People's Republic of China (COPR, 1981), and Indonesia (Iwaki et al., 1975). However, there is no report of CABMV naturally infecting cowpeas in the U.S. (Kuhn, 1990).

CABMV is of economic importance since it causes crop losses from 15 to 87 per cent under natural field conditions and from 29 to 44 per cent under artificial inoculation conditions (Kaiser and Mossahebi, 1975). Complete loss of an irrigated cowpea crop in northern Nigeria was recorded due to cowpea aphid-borne mosaic virus disease (Raheja and Leleji, 1974).

b. Viral molecular and/or particle characteristics, purification, and relationship with other potyviruses:

CABMV is characterized by flexuous filamentous particles ranging from 740 nm (Behnckn and Malveesky, 1977) to 750 nm (Bock, 1973) in length. The virus is classified as a member of the potyvirus group (Harrison et al., 1971; Fenner, 1976; Matthews, 1979; Hollings and Brunt, 1981)). Coat protein constitutes 95 % of the virion. The molecular weight of the subunit protein is 29,000 to 34,000 d (Edwardson and Christie, 1986). Nucleic acid is single stranded, positive sense RNA. CABMV isolates differ somewhat in stability, but in cowpea sap the thermal inactivation point lies between 57 - 60 C, the dilution end point lies between 10^{-3} and 10^{-4}, and infectivity is retained at 20 C for 1-3 days. Frozen infected leaves retain infectivity for at least 7 weeks (Bock, 1973; Lovisolo and Conti, 1966). The sedimentation coefficient (S 20, w) is 150 S for unaggregated particles (Bock and Conti, 1974).

Several purification procedures have been reported for CABMV (Bock, 1973; Ross, 1967; Taiwo et al., 1982; Bock and Conti, 1974). The virus can be propagated either on cowpea or *Nicotiana clevelendii* for purification purposes. Two to three weeks after inoculation, the systemically infected leaves are the best for purification of CABMV.

CABMV is moderately to strongly immunogenic (Bock, 1973). Among the African strains, the neotype and the mild strains are serologically identical, but the vein-banding strain is distinguishable, although related. European strains were considered to be similar based on a distant serological relationship with bean common mosaic virus (BCMV) (Lovisolo and Conti, 1966, Bock, 1973). Within the potyvirus group, CABMV was considered distantly related to BCMV and apparently shares no antigens with bean yellow mosaic virus (BYMV), pea seed-borne mosaic virus (Psbmv), clover yellow vein virus (CYVV), soybean mosaic virus (SbMV), potato virus Y (PVY), tobacco severe etch (TSEV), sugarcane mosaic virus (SCMV), or iris mosaic virus (IMV) (Bock, 1973). Some CABMV isolates did not show serological relationships with BCMV (Kaiser and Mossahebi, 1975; Fischer and Lockhart, 1976). There has been considerable confusion over the relationship between BlCMV and CABMV. The type isolate of CABMV (Lovisolo and Conti, 1966) was lost and some isolates previously assigned to that virus (e.g. CABMV isolates from Kenya and Nigeria) were identified as BlCMV (Taiwo, et al., 1982; Taiwo, and Gonsalves, 1982). However, a virus from Morocco has been referred to by Fischer and Lockhart (1976) as CABMV. The two viruses were shown to differ in host range and are regarded as distinct potyviruses (Lima et al., 1979; Taiwo, et al., 1982; Taiwo and Gonsalves, 1982: Shukla et al.,

1991). Viral isolates thought to have some properties similar to those of CABMV but not fully characterized include the cowpea mosaic virus of McLean (1941), Yu (1946), Van Velsen (1962), Shankar et al. (1973) and asparagus bean mosaic virus of Snyder (1942).

c. Viral host range and virus-induced symptoms:

Vigna unguiculata cultivars are the primary natural hosts but CABMV has been reported to infect 82 species in 46 genera of 13 families, including 53 species in 28 genera of the Leguminosae (Edwardson and Christie, 1986).

The nature and severity of the symptoms induced by CABMV vary with host cultivar, virus isolate, and the time of infection. Diseased plants show varying amounts of green vein-banding, interveinal chlorosis, leaf distortion, blistering, and stunting (Bock and Conti, 1974). Seed-borne infection usually is expressed in the primary leaves showing vein-clearing, vein-yellowing, diffuse chlorotic spots or patches, or intense chlorosis (Phatak, 1974). Later the symptoms are more distinct in trifoliate leaves. Long-term effects of infection are typically marked stunting, reduction in leaf area and delayed senescence. Some isolates of CABMV (e.g Morocco and PI-39C) quickly kill the whole plant under greenhouse conditions.

d. Viral strains/variants:

Five strains of CABMV have been described on the basis of symptomatology in cowpea cultivars. The European (type)

strain, which no longer exists, caused severe distorting mosaic (Lovisolo and Conti, 1966). The African (neotype) strain induces irregular, angular broken mosaic, and broad dark-green vein-banding (Bock, 1973). The African mild strain induces a very mild mottle, with little or no effect on plant growth (Bock, 1973). The Morocco strain which, though typically virulent, is now regarded as the tentative type strain of CABMV causing a mosaic pattern, leaf bumping, distortion and stunting according to Fischer and Lockhart (1976).

e. Relation of virus (and viral products) to host cells:

Cytoplasmic cylindrical inclusions consisting of pinwheels and bundles associated with scrolls are induced by CABMV (Lima et al., 1978; 1981). CABMV-induced cylindrical inclusions have the same morphology as those induced by BCMV and BlCMV. CABMV has been classified as a member of sub-division-1 of potyviruses (Edwardson and Christie, 1986).

f. Transmission through seeds and by vectors:

It is well established that the level of CABMV seed transmission varies among cultivars and is reported to range from 0 to 40 per cent (Bock and Conti, 1974; Kaiser and Mossahebi, 1975; Ladipo, 1977; Aboul-Ata et al., 1982; Phatak, 1974). Some cowpea lines possessing resistance to seed transmission have been identified (Ladipo, 1977; Mali

et al., 1983), although this form of CABMV control is exceeded by resistance to infection (see below).

CABMV is readily transmitted by sap inoculation and non-persistently by aphids (Atiri, 1982). *Aphis craccivora, A. gossypii, A. medicagenis, A. fabae, Macrosiphum euphorbie, and Myzus persicae* are reported to be vectors of CABMV (Vidano and Conti, 1965; Bock, 1973; Atiri et al., 1984). Aphids can retain infectivity up to 15 hr after virus acquisition (Bock and Conti, 1974). Cowpea cultivars possessing resistance or tolerance to *A. craccivora* have been identified (Chari et al., 1976; Singh and Allen, 1979, 1980).

g. Priority control measures:

Sources of resistance to CABMV infection have been reported (Ladipo and Allen, 1979; Taiwo, et al., 1982; Williams, 1977; Mali, et al., 1981; Mali, et al., 1988 and Patel et al., 1982; Bashir and Hampton, 1992). Three types of resistance are recognized: immunity, in which inoculated plants remain symptomless and from which virus cannot be detected or recovered; hypersensitive resistance, indicated by the development of necrotic localized lesions without systemic spread; and viral tolerance, in which systemic infection and normal viral replication occur with slight or no symptoms. Patel et al. (1982) reported that immunity to CABMV was controlled by a recessive gene in

association with minor/modifier genes, while the resistant reaction was governed by a partially dominant gene. Comparison of the reactions of certain cultivars to CABMV isolates showed pathogenic variation (Taiwo, 1978; Ladipo and Allen, 1979; and Patel et al., 1982). However, certain cowpea cultivars including TVU 2480 possess resistance effective against diverse isolates of CABMV (Allen, 1983). Two TVU lines TVU 401 and TVU 1582 have been identified to be immune to 50 CABMV isolates (Bashir and Hampton, 1992).

CHAPTER-1

SURVEY FOR SEED-BORNE POTYVIRUSES IN COWPEA IN SELECTED AREAS OF PUNJAB AND NWFP OF PAKISTAN

ABSTRACT

One hundred fifty-one leaf samples with virus-like symptoms were collected from 13 districts of Punjab and North West Frontier Province (NWFP) of Pakistan during the summer season of 1990 and 1991. Desiccated samples were tested by DAC and DAS-ELISA for the possible presence of seven viruses known to be seed-borne in cowpea: BlCMV, CABMV potyviruses, CMV cucumovirus, CPMV, CSMV comoviruses, CPMoV carmovirus and SBMV sobemovirus. The following viruses previously unreported in Pakistan-grown cowpeas were detected in one or more field samples: BlCMV (8%), CABMV (29%), CSMV (16.5%), SBMV (21.2%) and CPMoV (3.3%). A majority of the samples contained infectious complexes of two or more viruses, with CABMV as the most frequently component. CMV and CPMV were not detected in any of the samples.

Seven seed lots of cowpea were collected from different localities and evaluated for seed-borne viruses. Only CABMV was found in four of seven, with a transmission rates of < 1 to 7 percent. Likewise, field collected seeds from a local cultivar containing four ELISA-detectable

seed-borne viruses (BlCMV, CABMV, CSMV, and SBMV) seed transmitted only CABMV (7% frequency). Although all viruses occurring in cowpea in Pakistan are not yet identified, the present results clearly indicate that BlCMV, CABMV, CPMoV, CSMV and SBMV occur naturally in cowpea. This is believed to be the first record of these viruses in Pakistan.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important soil improving legume crop and is grown as a vegetable, a food grain and also for fodder purposes in Pakistan. It is grown as both spring and summer-season crops and is very popular in rainfed area of Punjab and North West Frontier Province (NWFP) due to its tolerance to drought. Cowpea is grown both alone as a food grain and combined with sorghum, maize or millet for fodder purposes. In irrigated areas of Punjab and NWFP, the crop is grown near the cities for vegetable purposes and intercropped with other summer vegetables. Recently, with the introduction of short season varieties, cowpea is becoming more important in irrigated and rainfed areas. Two seed-types of cowpea are popular in Pakistan: the small seeded, grey or green type, and the medium size, blackeye-type. The small seeded type is favored by the rural population, while the blackeye

type is preferred among the urban people.

Punjab province contributes about 80% of the total cowpea production in Pakistan, while the remainder comes from NWFP or Sind provinces. The average farm yield (450 kg/ha) represents 20% of the 2200 kg/ha produced at experimental stations. Viral diseases of cowpea are major constraints to high yields. Previously, only a whitefly (*Bemisia tabaci*) that transmits yellow mosaic virus (YMV) has been reported to naturally infect cowpea in Pakistan (Ahmad, 1978). However, several viruses have been reported in India where climatic conditions for cowpea cultivation are similar to those of Pakistan. Reports include BlCMV (Mali and Kulthe, 1980a, Mali et al., 1988, Mali, et al., 1990), CABMV (Mali et al., 1981, Mali, 1988), CMV (Sharma and Varma, 1975), SBMV (Singh and Singh, 1974), cowpea golden mosaic virus (Mali and Kulthe, 1980b), and BCMV (Sachchidanand et al., 1973). It would, therefore, seem logical to expect such viruses in cowpeas grown in Pakistan.

Seed-borne viruses have probably occurred for centuries in cowpea-growing areas of the world. However, they are becoming more important, in part perhaps due to the introduction of germplasm from regions where the viruses are indigenous. Despite of the prevalence and severity of cowpea viruses in Punjab and NWFP, there had been no information on the distribution and identification

of these viruses, prior to this study. A preliminary report of this investigation was published in 1991 (Bashir and Hampton, 1991).

MATERIAL AND METHODS

Thirteen districts, eight of Punjab and five of NWFP, each with half hectare or more commercial or experimental plots of cowpeas were surveyed during the summer seasons of 1990 and 1991. A total of 93 fields of commercial or experimental plots of cowpea were surveyed, and 151 leaf samples showing virus-like symptoms were collected from 13 districts of Punjab and NWFP. When sampled, the cowpea plantings ranged from seedling to podding stage depending on the sowing time at different localities/ altitudes. Leaf samples were taken at random from plants showing virus-like symptoms such as mosaic, mottle, or leaf distortion. Leaves from plants showing similar symptoms were considered as one sample. Samples were placed individually in moistened paper towels and transported in an ice chest to a refrigerator (4 C). All samples were desiccated over magnesium perchlorate in vacuum desiccators, and were brought to the Virology Laboratory, Department of Botany and Plant Pathology, Oregon State University, with quarantine permits issued by the USDA Animal and Plant Health Inspection Service and by the Oregon Department of

Agriculture. The samples were then processed for identification of viruses. Cowpea seed samples (200 to 500 seeds/sample) also were collected from local markets and from field grown plants during the survey to identify and investigate seed transmission of these seed-borne viruses.

Since double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and direct antigen coating (DAC) ELISA are sensitive techniques to detect plant viruses, these two serological tests were conducted to test the 151 leaf samples. Desiccated sample tissues were homogenized in antigen buffer (0.01 g/ml buffer) and processed by SAC or DAS-ELISA. Antigen buffer consisted of phosphate buffered saline with Tween 20 pH 7.2, containing 0.02 M sodium diethyldithiocarbamate (NaDIECA). Samples were tested for viruses by Enzyme-linked Immunosorbent Assay (ELISA), as the direct antigen coating (DAC) version. Following antigen coating virus specific purified immunoglobulin or crude antisera, the detection of antigen-antibody complexes was achieved with anti-rabbit IgG-conjugate. Antisera requiring cross-absorbance were typically diluted 1000 X with filtered homogenized healthy cowpea tissue in an antigen buffer (W/V, 500 X), incubated 1 hr at 37 C immediately prior to coating ELISA plates (Hampton et al., 1992a).

Double antibody sandwich ELISA (DAS-ELISA), antigen buffer, washing buffer and substrate buffer were prepared

as described by Clark and Adams (1977). The seven antisera or purified IgGs that were available in our laboratory (BlCMV, CABMV, CMV, CPMV, CPMoV, CSMV and SBMV) were used to test samples of tissue collected in Pakistan.

Seed samples were tested for seed-borne viruses by the growing-on test. Some 200 - 450 seeds of each sample were grown under insect-free greenhouse conditions. Seed infection was determined by symptoms induced on the primary and first trifoliate leaves, after which leaf tissues were tested by ELISA against seven viruses.

RESULTS

The number of fields visited and the number of samples collected from various districts of Punjab and NWFP have been shown in Table 1.1. Of 151 samples tested against seven antisera/IgGs of cowpea viruses, 71 samples reacted positively to one or more antisera/IgGs (Table 1.2). Of the five viruses detected, CABMV was the most prevalent. Forty-four out of 151 of the tissue samples contained CABMV either alone or in combination with one or more other viruses (Table 1.2). CABMV was detected in samples collected from four districts, Sialkot and Rawalpindi of Punjab, and Mansehra and Swat of NWFP. The next most frequently detected viruses were SBMV (23.8%) and CSMV (19.2%). SBMV was detected either alone or in mixed

Table 1.1: Number of cowpea fields surveyed and samples collected from various districts of Punjab and NWFP of Pakistan.

Province	District	No. of fields sampled during 1990	1991	Total fields sampled	No.of samples collected during 1990	1991	Total samples collected.
Punjab	Chakwal	2	3	5	4	3	7
	Faisalabad	3	3	6	6	10	16
	Gujranwala	3	2	5	3	2	5
	Lahore	6	3	9	8	5	13
	Rawalpindi	3	3	6	8	5	13
	Sargodha	5	2	7	5	3	8
	Sialkot	4	8	12	8	16	24
	Okara	-	1	1	-	2	2
NWFP	Balakot	2	3	5	3	4	7
	Haripur	2	2	4	3	2	5
	Mansehra	12	8	20	19	16	35
	Noshera	-	4	4	-	4	4
	Swat	6	3	9	6	6	12
	Total:	48	50	93	73	78	151

Table 1.2: Number of test samples from each district and their reactions in ELISA tests with one or more than one antisera/IgGs of viruses seed-borne in cowpea.

Province	District	No. of samples tested	Antisera to cowpea viruses						
			BlCMV	CABMV	CMV	CPMV	CSMV	SBMV	CPMoV
Punjab	Chakwal	7	-	-	-	-	-	-	-
	Faisalabad	16	-	-	-	-	-	-	-
	Gujranwala	5	-	-	-	-	-	-	-
	Lahore	13	-	-	-	-	5	5	2
	Rawalpindi	13	-	-	-	-	-	-	-
	Sargodha	8	-	-	-	-	-	-	-
	Sialkot	24	12	16	-	-	12	15	3
	Okara	2	-	-	-	-	-	-	-
NWFP	Balakot	7	-	-	-	-	1	1	-
	Haripur	5	-	-	-	-	-	-	-
	Mansehra	35	-	18	-	-	5	8	-
	Noshera	4	-	-	-	-	-	-	-
	Swat	12	-	7	-	-	2	3	-
	Total:	151	12	44	-	-	25	32	5

infection with other viruses, while CSMV was always found in mixed infection. Eight per cent of the samples contained ELISA-detectable BlCMV, and all these samples were collected from Sialkot (Punjab). Five samples (3.3%) contained of cowpea mottle carmovirus, detected by use of bean mild mosaic carmovirus (BMMV) antiserum. CMV and CPMV were not detected in any of the samples.

In most of the commercial cowpea fields near Sialkot (Punjab) and Buttle (Mansehra-NWFP), more than 80% of the plants showed virus-like symptoms. Most tissue samples from these fields contained two or more viruses (Table 1.3 & 1.4). Twenty-four samples from Sialkot District (Punjab) when tested against seven antisera/IgGs of cowpea viruses 12, 16, 12, 15 and 3 samples reacted positively with BlCMV, CABMV, CSMV, SBMV and CPMoV respectively. The A_{405} values recorded 90 min after addition of substrate (p-nitrophenyl phosphate) are shown in Table 1.3. Seven samples did not show any reaction to any of the seven antisera/IgGs tested.

Only CABMV was detected in the seven cowpea seed-lots tested for seven seed-borne viruses. Seed transmission rate of CABMV varied from < 1 to 7 per cent (Table 1.5). Field-collected seeds from a virus-infected, local, small-seeded cultivar were also tested for seed-borne viruses. Whereas the mother plants were determined to be infected with BlCMV, CABMV, CSMV and SBMV, only CABMV was seed-transmitted (7% frequency)

Table 1.3: A_{405}values produced by extracts from virus-infected cowpea samples collected from commercially grown fields of Sialkot District (Punjab), when tested against seven antisera/IgGs of cowpea seed-borne viruses.

Sample	A_{405}values recorded 90 min after incubation with substrate						
	BlCMV	CABMV	CMV	CPMV	CSMV	SBMV	CPMoV
S-1	0.027	0.012	0.001	0.001	0.003	0.004	0.012
S-2	0.005	0.004	0.034	0.002	0.025	0.007	0.031
S-3	0.021	0.023	0.032	0.040	0.007	0.061	0.023
S-4	1.707	0.302	0.012	0.034	2.577	0.385	0.012
S-5	0.012	0.004	0.041	0.007	0.017	0.001	0.004
S-6	0.007	0.412	0.002	0.021	0.031	0.019	0.011
S-7	0.653	1.211	0.017	0.018	2.023	1.021	0.002
S-8	0.753	1.009	0.009	0.015	1.315	1.209	0.001
S-9	0.320	1.070	0.034	0.017	2.227	1.715	0.697
S-10	0.771	0.494	0.028	0.003	0.521	1.315	0.002
S-11	1.112	0.574	0.009	0.013	0.424	>3	0.001
S-12	0.410	0.721	0.018	0.005	0.509	1.871	0.208
S-13	0.917	1.116	0.042	0.012	1.324	0.405	1.061
S-14	0.352	0.644	0.061	0.035	1.118	2.710	0.012
S-15	0.007	0.753	0.049	0.010	2.721	1.111	0.001
S-16	0.005	0.670	0.003	0.001	1.117	0.405	0.017
S-17	0.007	1.295	0.051	0.071	0.945	1.105	0.005
S-18	0.723	1.140	0.019	0.002	0.002	0.661	0.013
S-19	0.812	1.065	0.051	0.004	2.210	1.117	0.009
S-20	0.417	0.613	0.047	0.011	0.005	0.779	0.011
S-21	0.004	0.012	0.072	0.006	0.005	0.001	0.022
S-22	0.012	0.006	0.005	0.012	0.006	0.011	0.010

Cont,d..

Table 1.3: (Cont,d..)

Sample	A_{405} values recorded 90 min after incubation with substrate						
	BlCMV	CABMV	CMV	CPMV	CSMV	SBMV	CPMoV
S-23	0.002	0.001	0.041	0.004	0.001	0.021	0.051
S-24	0.001	0.024	0.071	0.002	0.002	0.041	0.011
Homolog.	1.204	1.004	2.002	NT	2.117	2.005	1.021
Healthy	0.003	0.006	0.003	0.002	0.001	0.002	0.012

NT = Not tested.
[1]Values are average of two wells.

Table 1.4: Mixed infection of cowpea viruses detected in field collected samples from Punjab and NWFP of Pakistan.

Province	District	Viruses found in mixed infection
Punjab	Sialkot	BlCMV/CABMV/CSMV/SBMV
	"	CABMV/CPMOV/CSMV/SBMV
	"	BlCMV/CABMV/CPMoV
	"	BlCMV/CPMoV/SBMV
	"	CABMV/CSMV/SBMV
	Lahore	CSMV/SBMV/CPMoV
NWFP	Balakot	CSMV/SBMV
	Mansehra	CSMV/SBMV
	"	CABMV/CSMV/SBMV

BlCMV = Blackeye cowpea mosaic virus
CABMV = Cowpea aphid-borne mosaic virus
CPMoV = Cowpea mottle virus
CSMV = Cowpea severe mosaic virus
SBMV = Southern bean mosaic virus

Table 1.5: Seed transmission of CABMV in seed lots of cowpea collected from different markets and field-grown plants in Punjab and NWFP.

Province	Location	Percent seed transmission	
Punjab	Ckakwal city (Chakwal)	0/285[1]	(0.0)[3]
	Narowal (Sialkot)	2/200	(2.0)
	Pattokey (Lahore)	11/431	(2.6)
	Texla (Rawalpindi)	2/238	(0.8)
	Sialkot (City)	9/127[2]	(7.0)
NWFP	Mangora (Swat)	0/215	(0.0)
	Khaza Khaila (Swat)	0/185	(0.0)

[1]Number of seedlings developed symptoms or reacted positively in ELISA /total seedlings examined.

[2]Seeds collected from virus-infected field-grown plants.

[3]values in parenthesis are percent seed transmission.

DISCUSSION

Under field conditions, multiple virus infection is usually more frequent than single infection. Multiple infections also modify symptom expression and make field diagnosis more difficult (Kuhn et al., 1966). When viruses occur in mixed infection, they may induce more severe symptoms due to a synergistic effect, and may cause high crop losses (e.g. BlCMV plus CMV). A yield loss of 10-42% was recorded in the case of mixed infection of BlCMV/CMV (stunt disease) or cowpea chlorotic mottle virus (CCMV)/SBMV on cowpea (Kuhn et al., 1966, Harrison and Gaudauskas, 1968).

Viruses were detected in 71 of 151 cowpea samples collected in 6 of 13 Districts of Punjab and NWFP CPMoV was detected in samples (3.3%) collected from Lahore and Sialkot districts of Punjab. CPMoV was found only in mixed infection with other viruses (Table 1.4). CPMoV previously had been reported only from Nigeria (Robertson, 1966; Rossel, 1977; Shoyinka et al., 1978). This is, therefore, the second report of CPMoV naturally infecting cowpea. Because CPMoV is seed-borne (Rossel, 1977), the virus may now be spreading to other parts of the world.

Eighty samples from plants with virus-like symptoms collected mostly from Lahore, Faisalabad, Okara, Gujranwala

and Sialkot districts of Punjab, did not react with any of the seven test antisera/IgGs. In Punjab, a whitefly (*Bemisia tabaci*) that transmits yellow mosaic virus (YMV) (not seed-transmissible) had been reported on cowpea (Ahmad, 1978). These 80 samples probably contained YMV or viruses other than the five detected. Although all the viruses occurring in cowpea in Pakistan have not yet been identified, the present results clearly indicate that BlCMV, CABMV, CPMoV, CSMV, and SBMV occur naturally in cowpea. Field occurrence of BlCMV, CABMV, CSMV, and SBMV have been reported either from India or Iran (Kaiser and Mossahebi, 1975; Rishi, 1988; Singh and Singh, 1974; Mali et al., 1988; Mali et al., 1990) which are neighboring countries of Pakistan, but this is believed to be the first report of BlCMV, CABMV, CPMoV, CSMV and SBMV in cowpea in Pakistan. Attempts to identify other cowpea viruses in Pakistan will be continued when the antisera to other cowpea viruses become available. Finally, it is proposed that all cowpea viruses presently in Pakistan be documented and that efforts be undertaken to generate virus-free seed for commercial cowpea production.

CHAPTER-2

DETECTION AND IDENTIFICATION OF SEED-BORNE POTYVIRUSES IN VIGNA PRE-INTRODUCTIONS AND GERMPLASM ACCESSIONS

ABSTRACT

Of 158 *Vigna unguiculata* pre-introductions being evaluated in the U.S. 1989-91, and being tested for seed-borne viruses under greenhouse conditions, 21 accessions representing eight countries of the world were found to be seed-infected with one or more of the three following viruses: BlCMV and CABMV potyviruses, and CMV cucumovirus. Seed transmission rates of 0-6.9%, 0-13.3%, and 0-2.0% were determined for BlCMV, CABMV, and CMV respectively. Twenty-two isolates of CABMV were collected and identified from *Vigna* germplasm nurseries (2930 total entries mostly from Botswana, Africa) grown under field conditions at the University of California, Riverside, California. BlCMV and CMV were also detected in samples collected from commercial and experimental plots at Riverside and the San Juaquin Valley of California. Overall, 43 isolates of BlCMV and 55 isolates of CABMV were derived from germplasm, seed lots, and field grown cowpea. Identities of BlCMV and CABMV isolates were determined by DAS-ELISA serology and verified by electron microscopy (EM) and host reactions.

INTRODUCTION

One of the most interesting characteristics of plant virus-host interactions is the high degree of protection against invasion by viruses from infected mother plants into embryos (Bennett, 1969). Nevertheless, some plant viruses are able to cross this barrier and perpetuate through seed from one plant generation to another. More than 200 plant viruses are reported to be seed-transmitted in one or more host species and the number of reports continues to increase (Mandahar, 1981, Hampton, 1983). There is now considerable circumstantial evidence suggesting that viruses have been introduced into many geographical regions by means of infected seed, and through increasing international exchange of germplasm for the purpose of crop improvement. Numerous instances of germplasm-borne viruses have now been reported, e.g. pea seed-borne mosaic virus (Psbmv) in pea (*Pisum sativum*) (Hampton and Braveman, 1979), PSbMV in lentil (*Lens culinaris*) (Hampton, 1982c), bean common mosaic virus (BCMV) and cucumber mosaic (CMV) in bean (*Phaseolus vulgaris*) (Klein et al., 1988; Davis et al., 1981), urdbean leaf crinkle virus (ULCV) in mungbean (*Vigna radiata*) (Beniwal et al., 1980), CABMV, CSMV, SBMV, and cowpea mild mottle virus (CMMV) in cowpea (Hampton, et al., 1992a), and BlCMV in yard-long bean or asparagus bean (*Vigna*

unguiculata ssp. *sesquipedalis*) (Dijkstra et al., 1987).

At least 16 viruses are reported to be seed-transmitted in cowpea (Hampton, 1983). The potyviruses reported in cowpea seeds are BlCMV (Zettler and Evans, 1977; Rishi, 1988; Mali et al., 1988; Mali et al., 1990; Dijkstra et al., 1987), CABMV (Hampton et al., 1992; Rishi, 1988; Mali et al., 1988; Ladipo, 1977; Phatak, 1974; Tsuchizaki et al., 1970b), BCMV (Sachchidanand et al., 1973), peanut mottle virus (PeMoV) (Demski et al., 1983). By means of seed transmission, viruses are randomly introduced throughout planted fields as foci of primary infections from which insect vectors can spread virus to healthy plants. Such inoculum is effectcious for disease epidemics because random inoculum distribution maximizes exposure of healthy plants to spread by vectors. If germplasm of a crop species is contaminated with seed-borne viruses, then breeding nurseries become recipients of this epidemic potential and viruses are passed to breeding progenies via seed transmission. Potyviruses are more dangerous as they are spread non-persistently by many species of aphids. This study was conducted to detect and identify the potyviruses that are seed-borne in *Vigna unguiculata*, pre-introductions and germplasm accessions and to collect a reasonable number of BlCMV and CABMV seed-borne isolates for comparisons of their serological and biological characteristics.

MATERIALS AND METHODS

Visit to University of California, Riverside:

During the summers of 1989 and 1990, we visited the Department of Botany and Plant Science, University of California, Riverside, California, where cowpea nurseries consisting of 744 and 1186 accessions respectively were planted under the supervision of Drs. T. Hall and P.N. Patel. The germplasm lines included in these nurseries were collected from African countries, particularly Botswana and Senegal. Farmer's fields and experimental plots of cowpea in the central San Juaquin Valley, Riverside, also were visited during those two years to collect potyvirus field isolates for these study. The surveys were conducted in late July or early August when cowpea plants were young so as to optimize opportunities to sample seed-borne viral inoculum. Diseased seedlings showing virus-like symptoms e.g. mottle, mosaic or leaf distortion, were collected in plastic bags, labelled as separate samples, and transported until processed for preservation and mechanical inoculation of glasshouse-grown plants. Half of each tissue sample was desiccated over magnesium perchlorate and stored at -20 C, while the other half was homogenized in buffer for ELISA serology and/or mechanical inoculation of healthy cowpea seedlings.

Evaluation of cowpea pre-introductions and germplasm accessions:

Seeds of 158 cowpea pre-introductions and germplasm accessions representing 12 countries of the world were obtained from the Southern Regional Plant Introduction Station, Griffin GA, U.S.A. Seeds of an additional 24 germplasm accessions were obtained from the Gene Bank, National Agricultural Research Center, Islamabad, Pakistan. Of these 182 accessions, seeds obtained for 73 were those imported from respective countries of origin. Seeds of the remaining 109 lines had been increased in the U.S.A. The country of origin and number of accessions from each country are indicated in Table 2.1.

About 200 seeds of each accession (10 seeds/pot 20 pots) were planted in clean plastic 8-inch pots filled with sterilized soil, and germinated/grown under insect-free greenhouse conditions. Seedlings were examined for symptoms of seed-borne viruses on primary or first trifoliate leaves (Figure 1). Seed-borne viruses were identified by DAS-ELISA tests of leaf tissue extracts, as described in Chapter-1. Anti-viral antisera/IgGs included BlCMV, CABMV, CMV, CPMV, CPMoV, CSMV, and SBMV. Identities of selected seed-borne isolates of BlCMV and CABMV were confirmed by electron microscopic examination of extracts from infected leaves stained in ammonium molybdate (2%), pH 7.0. Flexuous rod shaped particles characteristic of

Table 2.1: Name of countries and the number of cowpea pre-introduction and germplasm accessions from each country tested for the detection of seed-borne potyviruses in cowpea.

Country	Accessions with original seed (no.)	Accessions with increased seed (no.)	Total accessions tested(no.)
Afghanistan	1	20	21
Botswana	9	0	9
Brazil	1	3	4
China	3	6	9
Hungary	6	4	10
India	5	16	21
Iran	10	10	20
Mexico	7	1	8
Nigeria	0	12	12
Pakistan	26	13	39
South Africa	4	6	10
U.S.A.	1	18	19
Total:	73	108	182

Figure 1. CABMV symptoms from seed-borne infection.

(a). Vein chlorosis symptoms induced in seedlings of PI 218123 by seed-borne CABMV isolate PI-42C.

(b). Systemic dark green vein banding symptoms induced in plants of PI 251222 by CABMV seed-borne isolate RN-7C.

potyviruses were observed. Selected isolates of BlCMV and CABMV were also tested against potyvirus selective monoclonal antibody II-197 (Wang et al., 1984). Plants being examined for seed-borne viruses were observed for 4 weeks to monitor delayed symptom development. Accessions tested for seed-borne potyviruses in this study are listed in Appendix-1.

Mechanical inoculation and maintenance of BlCMV and CABMV potyvirus isolates:

Isolates of BlCMV and CABMV derived either from seeds or field samples were mechanically inoculated to susceptible cowpea lines PI 218123 or PI 251222 (susceptible to almost all BlCMV and CABMV isolates). For virus propagation, carborundum-dusted (600 mesh), fully expanded primary leaves of susceptible seedlings were inoculated with extracts from virus-infected leaves homogenized in buffer (0.02 M phosphate buffer, pH 7.0). All isolates were maintained on susceptible cowpea lines under insect-free greenhouse conditions.

Precautions were taken to protect the purity of viral isolates maintained in glasshouses. These included (a) separation of cowpea plants infected with individual isolates, (b) carefully planned and regular applications of insecticides, (c) careful avoidance of needless touching of infected plants and thorough cleaning of hands when transferring and maintaining viral isolates, and (d)

disinfection of glasshouse tools with tri-basic sodium phosphate. Additionally, reserve sources of each isolate were maintained at - 30 C in desiccated, infected cowpea tissues. BlCMV and CABMV isolates were occasionally discovered to have become contaminated with CMV, whereupon pure isolates were re-established from their respective reserve sources. BlCMV and CABMV isolates derived from seed or field-plant samples and included in these studies are indicated in Appendix-2.

RESULTS AND DISCUSSION

Twenty-two isolates of CABMV were collected from 1781 pre-introductions/germplasm accessions during 1989 and 1990, grown at the University of California, Riverside, (see Appendix-2). Infected seedlings in nursery plots showed very characteristic CABMV-like symptoms. All 22 isolates reacted positively against anti-CABMV immunoglobulin (IgG), and reacted negatively with five antisera/IgGs to other cowpea viruses, BlCMV, CMV, CSMV, CPMoV and SBMV (Table 2.2). No mixed infection of other viruses was detected in any of these samples. Based on serology, particle morphology, and host reactions, all of these isolates were identified as CABMV isolates. One isolate, RF-4B, also isolated in 1989 from field-grown cowpea plants (i.e. Poplar plots, Tulare County, California), reacted

Table 2.2: Preliminary DAC/DAS ELISA results of CABMV isolates collected from *Vigna* germplasm nurseries grown in University of California, Riverside, during 1989 and 1990.

Isolate	A_{405nm} values 90 min after incubation with substrate					
	DAS-ELISA[1] (IgG)		DAC-ELISA[2] (Antiserum)			
	BlCMV	CABMV	CMV	CSMV	SBMV	CPMoV
RN-2	0.013[3]	0.778	0.010	0.001	0.003	0.013
RN-3	0.001	1.427	0.001	0.003	0.004	0.014
RN-4	0.003	1.785	0.013	0.016	0.023	0.020
RN-5	0.010	1.869	0.004	0.021	0.010	0.022
RN-6	0.014	0.901	0.011	0.041	0.007	0.020
RN-7	0.031	1.472	0.005	0.006	0.027	0.010
RN-10	0.014	0.979	0.006	0.071	0.019	0.005
RN-11	0.006	1.427	0.023	0.016	0.042	0.041
RN-12	0.005	1.290	0.019	0.009	0.023	0.021
RN-13	0.012	1.322	0.006	0.001	0.006	0.010
RN-14	0.019	1.322	0.007	0.000	0.001	0.002
RN-15	0.007	1.429	0.021	0.031	0.009	0.009
RN-18	0.004	1.817	0.010	0.040	0.071	0.013
RN-27	0.020	0.219	0.013	0.003	0.012	0.017
RN-28	0.014	0.527	0.006	0.006	0.004	0.055
RN-29	0.010	1.004	0.006	0.028	0.001	0.041
RN-30	0.050	0.706	0.015	0.017	0.003	0.003
RN-31	0.020	0.675	0.007	0.070	0.041	0.010
RN-34	0.010	1.704	0.019	0.010	0.021	0.018
RN-35	0.002	1.107	0.022	0.003	0.007	0.007
RN-36	0.020	1.638	0.031	0.001	0.013	0.034
RN-37	0.018	1.464	0.008	0.009	0.001	0.004
RN-38	0.011	1.976	0.000	0.005	0.031	0.013
RF-4B	1.503	0.013	0.041	0.016	0.027	0.012
Homologous	1.823	1.760	1.332	2.141	1.923	1.337
Healthy	0.004	0.007	0.039	0.002	0.016	0.003

[1]: Each antigen (isolate) was diluted in virus extraction buffer (VEB) (PBS-Tween-PVP), virus specific antibody-conjugate diluted to 1000 X in VEB. [2]: Antiserum dilution was 1000 X for DAC with cross absorbance in 500 X healty sap, antirabbit IgG-conjugate was diluted to 5000 X. [3]: Values are mean absorbance of two wells for each antigen (isolate),

exclusively with anti-BlCMV IgG (Table 2.2), and therefore was identified as a BlCMV isolate. Twelve samples collected from symptomatic cowpea plants in experimental entomology plots at the Kearny Station, Tulare County, California, contained pure CMV as determined by DAS-ELISA. No CABMV was detected in any sample collected from commercial fields or experimental plots in southern California.

Of 182 seed lots of cowpea pre-introductions/ germplasm accessions evaluated for seed-borne viruses, 21 accessions were found to contain one of the following viruses: BlCMV or CABMV potyvirus, or CMV cucumovirus (Table 2.3). CMV was detected only from accessions that originated in Iran. Seed transmission rates of 0-6.9, 0-13.3, and 0-2.0% respectively, occurred for BlCMV, CABMV and CMV. No mixed infection of two or more viruses was detected.

Three viruses, BlCMV, CABMV, and CMV were detected from a sample of 158 pre-introduction accessions maintained in the Southern Regional Plant Introduction Station, Griffin GA U.S.A. BlCMV was detected from accessions originating from India, Brazil, Hungary, Afghanistan, and Pakistan. BlCMV has been reported in cowpea seeds from India (Rishi, 1988; Mali et al., 1990, Hampton et al., 1992a) and Brazil (Lin et al., 1981), but not from Hungary and Afghanistan. Recently, BlCMV has been reported from

Table 2.3: Vigna pre-introductions found infected with seed-borne BlCMV and CABMV potyviruses and CMV cucumovirus.

PI Number	Country of origin	Seed Status	Percent seed transmission
A. Accessions found infected with BlCMV.			
PI 353303	India	Original	3.0
PI 352830	India	Increased	1.0
PI 269664	India	"	1.4
PI 352839	India	"	6.9
PI 487554	Brazil	"	1.4
PI 292902	Hungary	"	1.3
PI 251222	Afghanistan	"	1.5
PI 218123	Pakistan	"	1.0
B. Accessions found infected with CABMV.			
PI 491454	Botswana	Original	0.5
PI 491450	Botswana	Increased	0.5
PI 218123	Pakistan	"	3.0
PI 220847	Afghanistan	"	3.0
PI 223720	India	"	2.0
PI 302847	U.S.A	"	13.3
C. Accessions found infected with CMV.			
PI 223420	Iran	Increased	1.2
PI 229735	"	"	0.5
PI 471442	"	Original	2.0
PI 417443	"	"	1.3
PI 471445	"	"	1.5
PI 491455	"	"	1.5
PI 491459	"	"	1.0

Pakistan (Bashir and Hampton, 1991). Seven accessions from Afghanistan, Botswana, Pakistan, India and, U.S.A. were found to contain seed-borne CABMV. Occurrence of CABMV in cowpea seeds in several African and other countries had been previously reported(Bock, 1973; Ladipo, 1976; Patel and Kuwite, 1982; COPR, 1981,; Fischer and Lockhart, 1976, Rossel, 1977), India (Mali and Kulthe, 1980a; Mali et al, 1988, Mali et al., 1990; Hampton et al., 1992a), and in Pakistan (Bashir, and Hampton, 1991). Although CABMV was not previously known to occur in the U.S., it was considered likely that U.S. germplasm collection of *Vigna unguiculata* would contain this and other exotic viruses (i.e., many accessions have been introduced from world regions were CABMV is indigenous in cowpea). This study provided an opportunity to test this idea. During the period of 1989-1992, we discovered three instances of natural CABMV spread from introduced cowpea accessions to indigenous U.S. Cowpeas (e.g., at Griffin, Georgia; Auburn, Alabama; and Rio Grand, Texas). In one instance, a U.S.-developed cowpea genotype was introduced into the U.S. *Vigna* germplasm collection, and was subsequently discovered in this study to have become infected with CABMV (i.e., PI 302287, 13.3% incidence of seed-borne CABMV, when examined by us in 1990). It is presumed that during its development this genotype had been exposed to, and infected from, introduced *Vigna* germplasm.

Previously stated natural occurrence of CMV on several legume crops in Iran has been reported (Kaiser et al., 1968).

The evaluation of 158 *Vigna unguiculata* accessions provided evidence that at least three seed-borne viruses, BlCMV, CABMV, and CMV, occur in cowpea germplasm seed stock in the U.S. It was once assumed that BlCMV was a virus occurring only in new world countries, and that CABMV occurred only in old world countries. This study, however, provides evidence to the contrary, e.g., BlCMV has been detected from accessions, originating in Afghanistan, India and Pakistan (Table 2.2). Likewise, CABMV has now been detected in seeds of a pre-introduction and in experimental field-grown plants, as previously mentioned. Some isolates from Africa (Bock, 1973), which were previously considered as CABMV, are now identified as BlCMV (Taiwo et al., 1982). These reports and our results indicate that BlCMV and CABMV can occur in the same geographical areas, wherever cowpea is grown in the world.

Significantly, *Vigna unguiculata* has the distinction of hosting more seed-borne viruses than any other crop species (Hampton, 1983). It is therefore likely that more seed-borne viruses will be detected in germplasm accessions with further investigation. Distribution of germplasm containing seed-borne viruses is dangerous to potential users and can result in introduction of exotic

viruses to new cowpea producing regions. Establishment and distribution of virus-free germplasm is the recommended control against germplasm-borne viruses. (Hampton et al., 1992c). ELISA is an excellent technique for the detection of seed-borne viruses. We therefore propose that more *Vigna* accessions of germplasm be assayed for important cowpea seed-borne viruses. Seed-borne viruses, when undetected and unrecognized, can accompany crop genes into breeding progenies. When this occurs, the viruses act as biological time bombs and threaten cultivar development, breeding materials and surrounding crops (Hampton et al., 1982b).

CHAPTER-3

PURIFICATION, ANTISERUM PRODUCTION AND ELECTRON MICROSCOPY OF A TYPICAL ISOLATE OF CABMV (RN-7C) SEED-BORNE IN COWPEA

ABSTRACT

Eight isolates of BlCMV and nine of CABMV were purified by three comparative potyvirus purification methods, including several host-isolate combinations. Average viral yields of 2.96 and 4.42 mg/100 g infected plant tissues were achieved for BlCMV and CABMV, respectively. Leaf tissue harvested two weeks after inoculation was found to be the most suitable for purification of BlCMV and CABMV isolates. Among the three methods of purification compared, methods M-II and M-III were determined to be the most effective, for purification of CABMV and BlCMV respectively. Carbon tetrachloride when used in combination with chloroform as a clarifying agent improved purification of most of the BlCMV isolates, but was harmful to CABMV isolates. Borate buffer proved to be superior over phosphate buffer for purification of isolates of both viruses. Addition of EDTA and Triton X-100 in the extraction and resuspension buffers circumvented virion aggregation. The A_{260}/A_{280} ratios for BlCMV and CABMV isolates agreed closely with published average values of 1.2.

Antiserum produced against a typical isolate of CABMV,

RN-7C, reacted positively with homologous and heterologous antigens producing ELISA A_{405} values that distinguished between extracts from CABMV-infected versus healthy plants. Both DAC-ELISA (using antiserum) and DAS-ELISA [using purified immunogammaglobulin (IgG)] were standardized to determine optimum concentration of reagents. The use of crude antiserum in DAC-ELISA or IgG in DAS-ELISA when diluted to 1:1,000 were found to be capable of detecting virus in fresh or desiccated infected tissues that were also diluted 1:1,000.

Leaf dip or purified preparations of BlCMV or CABMV isolates, when examined under an electron microscope, were found to contain elongated flexuous particles with modal length of 742 nm, typical of potyviruses.

INTRODUCTION

Potyviruses are often difficult to purify because of their tendencies towards irreversible aggregation during extraction and concentration with consequent loss during low speed centrifugation. Several protocols have been described for the purification of BlCMV and CABMV (Lima et al., 1979; Purcifull and Gonsalves., 1985; Zaho et al., 1991b; Ross, 1967, Bock and Conti, 1974).

CABMV isolate RN-7C was collected from a germplasm nursery, University of California, Riverside, during 1989.

Subsequent studies suggested that RN-7C was typical and representative of CABMV; thus it was selected as a tentative type isolate. The origin of germplasm from which RN-7C was isolated was Botswana, where CABMV is a predominant virus of cowpea. The specific objectives of the following study were (a) to standardize the purification method for BlCMV and CABMV, to optimize yield of virus for use in other studies (b) to produce antiserum against CABMV (RN-7C), for detection/ identification of other CABMV isolates and (c) electron microscopy to visualize particle morphology and to measure virion size. Purification procedures, electron microscopy, and standardization of direct (DAS) and indirect (DAC) ELISA methods are presented here.

MATERIALS AND METHODS

Virus isolates:

Details concerning eight isolates of BlCMV and nine of CABMV including RN-7C propagated and purified are presented in Appendix-2.

Virus propagation:

RN-7C and other isolates of BlCMV and CABMV were propagated on cowpea lines/cv, PI 218123, PI 251222 or Pusa Phalguni, susceptible to almost all viral isolates. Plants were inoculated by rubbing carborundum-dusted fully-

expanded primary leaves with extracts from plants infected by individual isolates. Inoculated plants were maintained in insect-free greenhouse conditions optimized for cowpea growth and development (i.e. temperature, 28-30 C; lights, 14 hr photoperiod; 125-174 langleys). Leaves from inoculated plants were harvested for purification, at time intervals ranging from 2 to 4 weeks after inoculation.

Purification:

Three methods were evaluated for purification of BlCMV and CABMV isolates. Initially CABMV isolate RN-7C was purified by Method I, below as described by Bock (1973) with some modifications, to produce antiserum against this isolate. Two other methods were subsequently evaluated, Method II and Method III, below.

Method 1:

Two weeks after inoculation systemically infected leaves were harvested and homogenized in 0.5 M phosphate buffer, pH 7.0 (1 g tissue/5 ml EB) containing 0.72 % 2 mercaptoacetic acid, 6% urea, 0.2% Triton X-100, 0.2% Antifoam B, 0.01 M sodium diethyldithiocarbamate (Na-DIECA) and 0.001 M $MgCl_2$. The slurry was clarified with chloroform (1 ml/ g tissue) and the aqueous phase subjected to a cycle of differential centrifugation (118,000 G and 7,000 G). The pellets were resuspended in 0.5 M phosphate buffer, pH 7.0, containing Triton X-100 and Na-DIECA, clarified by low speed centrifugation, and layered on

10-40% sucrose density gradients prepared in the above phosphate buffer. Virus fractions were collected by running the gradient columns on ISCO fractionator and concentrated by ultracentrifugation (118,000 G). The final pellet was suspended in 0.01 M phosphate buffer, pH 7.0. Virus concentration was estimated spectrophotometrically with an extinction coefficient of 2.4 (1 mg/ml) cm^1 at 260 nm after correction for light scattering. An A_{260}/A_{280} ratio of 1.2 was used as an index of viral purity (Lima et al., 1979).

Method II:

This method of purification was basically the same as described by Hampton et al. (1992b). The virus was extracted from prechilled systemically infected cowpea leaves homogenized with cold buffer A [0.2 M borate buffer, pH 8.0, containing 0.5% mercaptoethanol, 1mM ethylenediaminetetraacetate (EDTA), 1% Triton X-100, 1% Antifoam B, and 20% (v/v) chloroform. The last three reagents were added just before use of the buffer. Homogenized tissue was subjected to low speed centrifugation (3,000 G). The supernatant liquid was filtered through glass wool, precipitated by stirring for 45 min with 6% polyethylene glycol (PEG), and centrifuged, 10 min, 16,000 G. The resulting pellet was resuspended (45 min, 4 C, with stirring) in cold buffer B (0.01 M borate buffer, pH 8.0, containing 0,05% mercaptoethanol, 1mM EDTA, and 1% Triton

X-100). The suspension was clarified by centrifugation, 10 min, 3,000 G, filtered through glass wool, centrifuged, 90 min, 118,000 G., and the pellet was suspended in buffer B. The suspension was layered on 10-40% sucrose density gradients (prepared in buffer B), and centrifuged, 150 min, 104,000 G. Bands associated with virions were manually collected, diluted 1:1 with buffer B, and centrifuged, 90 min, 118,000 G. The final pellet was suspended in buffer C (0.01 M borate buffer , pH 8.0, without any additives), and the virus concentration was estimated spectrophotometrically, using an extinction coefficient of 2.4.

Method III:

In the course of developing this method, elements of several published methods were evaluated, including Lima et al. (1979); Taiwo et al. (1982); and Zaho et al. (1991). None of several efforts resulted in success, primarily because of virion aggregation. The protocol outlined below was essentially a modification of Method II and was satisfactory for BlCMV and with modification, also for CABMV.

Systemically infected cowpea leaves were harvested two weeks after inoculation, and homogenized in extraction buffer (EB), 0.2 M borate buffer, pH 8.0, containing 0.75% 2 mercaptoethanol), 0.001 M ethylenediamine tetraacetic acid (EDTA), and 1% Triton X-100 (alkylphenoxy polyethoxyethanol). The plant extract was clarified with

chloroform (20% volume of EB) and carbon tetrachloride (10% volume of EB), precipitated with 5% polyethyleneglycol and resuspended. The suspension underline with a 40% sucrose cushion was subjected to ultracentrifugation, 90 min, 118,000 G. Resulting pellets were resuspended overnight in 0.01 M borate buffer, pH 8.0 containing, 2 ME, EDTA and Triton X-100. The suspension was layered onto 10-40% sucrose density gradients, centrifuged 150 min, 104,000 G. Virus bands were manually collected from the gradient columns by syringe and concentrated by centrifugation (118,000 G, 90 min). The final pellet was resuspended in 0.01 M borate buffer pH 8.0, and the virus concentration was estimated.

Production of antiserum:

Antiserum against CABMV isolate RN-7C was prepared by injecting a male New Zealand White rabbit with 250 ug of purified virus preparation. Initially the virus was emulsified with 1 ml Freund's Complete Adjuvant and RIBI (Monophosphoryl lipid A (PML™) Immuno Chem, Research Inc. U.S.A), whereas, three subsequent injections of 600 ug purified virus were prepared in equal portions of complete and incomplete Freund's Adjuvant in equal ratio and injected at weekly intervals. The first bleeding was taken 3-weeks after the first injection, followed by three more bleedings at two-week intervals. All the bleedings were taken aseptically by cardiac puncture. The blood was

allowed to clot at room temperature for 1-2 hr before being loosened from the flask by agitation, and stored at 4 C overnight to allow maximum clot shrinkage and serum yield. Subsequent antiserum collection was repeated for two days until maximum serum yield was obtained. Serum was removed with a sterile pipet, centrifuged for 10 min at 1,700 G, and 0.01% sodium azide (NaN_3) was added to the antiserum as a bactericide before storage at -20 C or 4 C.

Standardization of antiserum and purified immunogamma-globulin (IgG) for DAC and DAS ELISA tests.

DAC-ELISA:

Two methods were followed for standardizing of DAC-ELISA procedures. These methods have been described in Table 3.1 & 3.2.

DAS-ELISA:

DAS-ELISA was performed basically as described by Clark and Adams (1977). Protocols for purification of immunogammaglobulin (IgG) from the crude antiserum, for enzyme-conjugation, and for standardized ELISA are outlined in Table 3.3, 3.4 & 3.5. IgG from crude antiserum was purified by sodium sulfate precipitation. Optional 96 well microtiter plates were Dynatech Immunolon II or Falcon 3915 Becton Dickinson & Co. New Jersey U.S.A. Completed ELISA reactions were read on Microplate Autoreader EL 309,

Table 3.1 : Method No.1, for standardization of DAC-ELISA using crude antiserum with blocking.

1. Add 200 ul antigen samples diluted (1:100) in virus buffer (PBS-Tween+0.2% egg albumin and 2% polyvinylpyrrolindone-PVP 10)
2. Incubate 2 hr at 37 C or over night at 4 C.
3. Rinse plate 3 times, 3 min each, with washing buffer (PBS[a] + 0.1% Tween-20 (Polyoxyethylene sorbitan monolaurate).
4. Add 200 ul blocking solution (0.1% dry milk dissolved in 1X PBS)in each well.
5. Incubate at 37 C for 1 hr.
6. Repeat step 3.
7. Add 200 ul antiserum diluted in virus buffer (1:500) in each well.
8. Repeat step 2.
9. Repeat step 3.
10. Repeat step 4, 5 & 6.
11. Add 200 ul of goat anti-rabbit IgG-conjugate diluted 1:1000[b] (v/v)in virus buffer.
12. Repeat step 2 & 3.
13. Add 200 ul p-nitrophenyl phosphate (1 mg/ml) in 9.7% diethanolamine, 0.2% NaN_3, pH 9.8) to each well.
14. Incubate for 1-2 hr and measure optical density (OD) at 405 nm.

[a]PBS: 0.137 M NaCl, 1.4 mM KH_2PO_4, 8 mM $NaHPO_4$, 2.7 mM Kcl pH 7.4 with 0.02% NaN_3.
[b]Optimum dilution determined by standardization trials may vary with each conjugate or gamma globulin produced.

Table 3.2: Method No.2 for standardization of DAC-ELISA using crude antiserum with cross absorbance.

1. Add 200 ul antigen samples diluted (1:100) in antigen buffer (1X PBS containing 0.23% Na-DIECA).
2. Incubate for 2 hr at 37 C or over night at 4 C.
3. Rinse plate 3 time, 3 min each, with washing buffer (as described in Table-1)
4. Dilute antiserum typically 1000 X with filtered of homogenized healthy cowpea tissue in virus buffer (w/v, 500 X), incubate 1 hr at 37 C immediately prior to add 200 ul to each well.
5. Repeat step 2.
6. Repeat step 3.
7. Add 200 ul antirabbit IgG-conjugate (1:1000) diluted in virus buffer.
8. Repeat step 2.
9. Repeat step 3.
10. Add 200 ul p-nitrophenyl phosphate substrate.
11. Incubate for 1-2 hr and measure optical density (OD) at 405nm.

Table 3.3: Protocol for preparation of IgG from crude antiserum produced against RN-7C isolate.

1. Combine 1.2 ml crude antiserum with 1.2 ml double distilled water.
2. Combine the 2.4 ml of antiserum-water 2.4 ml of 36% sodium sulfate (Na_2SO_4, dissolved in double distilled water). Vortex mixture and let it stand for 10 min at room temperature.
3. Centrifuge at 22 C for 15 min at 12,000 g. Decant supernate, and save pellet of white precipitate.
4. Add 10 ml of 18% sodium sulfate, and vortex.
5. Repeat step 3, and save pellet.
6. Resuspend pellet in 1 ml of PBS. Dialyze against 1 liter 1X PBS, at 4 C, changing buffer after 1 hr; again after 2 hr and 16 hr.
7. Examine preparation for visible precipitate; remove any precipitate by centrifugation , 12,000 g 15 min.
8. Estimate IgG concentration by absorbance at 280 nm, assuming extinction coefficient of 1.4 (i.e. 1mg/ml, where A_{280} of stock divided by 1.4= total 1 mg IgG in 1 ml stock).

Table 3.4: Protocol for preparation of IgG-enzyme (alkaline phosphatase) conjugate.

1. Dilute IgG stock with 1X PBS to produce 1.0 ml at a concentration of 1.0 mg/ml (A_{280}=1.4)
2. Add this 1.0 ml of IgG to 1000 units of lyophilized alkaline phosphatase and vortex gently.
3. Dialyze mixture against one liter 1X PBS, changing buffer after 1 hr, 2 hr, and 16 hr.
4. Measure volume of the dialyzed mixture and transfer to a small glass tube.
5. While gently vortexing the mixture add fresh glutaraldehyde to a concentration of 0.2% (i.e., add 8 ul of glutaraldehyde to stock per ml of mixture). Vortex until homogenized.
6. Repeat dialysis as described in step 3, above.
7. Store concentrated IgG at 4 C.
8. Dilute concentrated IgG in 1X PBS to get A_{280} 1.4 (i.e., 1mg/ml), to be used for coating microtiter plates.

Table 3.5: Protocol for DAS-ELISA.

1. Add 200 ul immunogammaglobulin diluted 1:1000[a] (v/v) in coating buffer (0.05 M sodium carbonate, 0.02% NaN_3 pH 9.6) to each well.
2. Incubate plate for 4-5 hr at 37 C or over night at 4 C.
3. Rinse plate 3 times, 3 min each, with PBS[b] (Table-1) + 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate)
4. Add 200 ul sample diluted in virus buffer (PBS-Tween + 0.2% egg albumin and 2.0% polyvinylpyrrolindane PVP 10)
5. Repeat step 2.
6. Repeat 3.
7. Add 200 ul enzyme-conjugate diluted 1:1000[a](v/v) in virus buffer.
8. Repeat step 2.
9. Repeat step 3.
10. Add 200 ul p-nitrophenyl phosphate substrate (1 mg/ml in 9.7% diethanolamine, 0.2% NaN_3, pH 9.8) to each well.
11. Incubate 1-2 hr, measure OD at 405 nm.

[a]Optimum dilutions determined by standardization trials may vary with each conjugate or globulin produced.

[b]PBS= 0.137 M NaCl, 1.5 mM KH_2PO_4, 8.0 mm $NaHPO_4$, 2.7 mM Kcl pH 7.4 with 0.02% NaN_3

BIO-TEK Instruments Inc., Winooski VT 05404 U.S.A.

Electron microscopy:

Leaf dip preparations or purified virus were applied to carbon-stabilized formvar-coated copper grids, negatively stained with 2% ammonium molybdate, pH 7.0 and examined by a Philips Model CM 12 transmission electron microscope and photographed. The particle size was estimated by comparing the projected micrographic virions with that of diffraction grating of the same magnification. About 100 particles of CABMV isolate RN-7C were measured. The virion length occurring most frequently was treated as its modal length (Walkey, 1985). The data were assembled and analyzed as a histogram, as outlined by Tomlinson (1964).

RESULTS

Virus propagation and purification:

Of several cowpea cultivars/lines tested for propagation of BlCMV and CABMV isolates, three cowpea genotypes, PI 218123, PI 251222 and Pusa Phalguni were found to be suitable for almost all of the isolates of BlCMV and CABMV. An exception was the type isolate, CABMV-Mor, which caused whole plant necrosis of PI 218123 seedlings, and this isolate was therefore propagated and maintained either on PI 251222 or Pusa Phalguni. *Nicotiana benthamiana* was considered alternatively suitable for

maintaining and propagation of some BlCMV and CABMV isolates.

Initially CABMV RN-7C isolate was propagated and purified using cowpea cultivars and following several protocols as described for this virus (Bock, 1973; Bock and Conti, 1974). Initial attempts resulted in poor virus yield and virion aggregation, however, sufficient quantities of purified virus were obtained for production of excellent antiserum to this isolate.

Variable yield of BlCMV and CABMV virions was observed with different combinations of host genotypes, virus isolates, and purification methods (Table 3.6). High yields were obtained (8 mg virus/50 g infected-plant tissues) when CABMV isolate RN-7C was purified from *N. benthamiana* three weeks after inoculation. However, poor yields (0.521 mg/ 50 g) were obtained when BlCMV isolate "Ga" was purified from the same host, by the same method. The yields of isolates purified from 50 g leaf tissue of Pusa Phalguni by Method II were 3.44, 3.02, 0.02, and 1.69 mg for CABMV isolates RN-10C, PI-23C, PI-42C2 and CABMV-Mor respectively, whereas the yields of the BlCMV isolates RF-4B, PI-24B, and PI-25B from the same host were 3.12, 2.58, 2.24 respectively by Method III. It was interesting to note a variability in virus yields not only between the two viruses, but also among the isolates of the same virus.

Table 3.6: Yield and $A_{260}/_{280}$ ratio of CABMV and BlCMV isolates purified from cowpea and tobacco (*Nicotiana benthamina*) by purification method No 1, II and III.

Isolate	Host/ genotyp	Method	Trials	Yield (mg/50g)	Yield range	$A_{260}/_{280}$
A. BlCMV isolates						
BlCMV-Ga	Tobacco	I	1	0.521	-	1.110
BlCMV-Ga	PI 251222	I	1	0.272	-	0.852
BlCMV-Ga	PI 251222	II	1	1.672	-	1.120
BlCMV-Ga	PI 251222	III	1	2.210	-	1.280
PU-1B	PI 251222	III	1	1.775	-	0.983
PU-7B	PI 218123	III	1	1.145	-	1.130
RF-4B	Pusa Phalu	III	1	3.120	-	1.190
PI-23B	PI 218123	III	1	0.538	-	1.450
PI-24B	Pusa Phalu.	III	2[a]	2.583	-	1.872
PI-25B	Pusa Phalu.	II	1	2.240	-	0.724
RF-26B	Pusa Phalu.	1	1	0.247	-	0.927

Cont,d..

Table 3.6 (Cont,d..)

Isolate	Host/ genotype	Method	Trials	Yield (mg/50g)	Yield range	$A_{260}/_{280}$
B. CABMV isolates						
9-7C	PI 218123	I	4	0.946[a]	0.17-2.50	1.230
9-7C	PI 218123	II	3	1.990	0.33-3.40	1.280
9-7C	PI 218123	III	2	0.575	0.41-0.74	0.875
9-7C	Tobacco	I	1	8.333	-	1.220
CABMV-Bz	PI 251222	I	1	2.720	-	0.851
PK-3C	Local	I	2	0.985	0.80-1.17	1.060
RN-10C	Pusa Phalu.	II	1	3.448	-	1.140
PI-23C	Pusa Phalu.	II	1	3.029	-	1.312
PI-39C	PI 218123	II	2	1.055	0.88-1.23	1.211
RN-34C	Pi 218123	II	1	0.732	-	0.754
PI-42C2	Pusa Phalu.	II	1	2.029	-	1.312
CABMV-Mor	Pusa Phalu.	II	1	1.697	-	1.070

[a]The number indicates the average value when the trials are more than one.

Among three purification methods tried, Method II and Method III worked well for CABMV isolates. Only Method III was suitable for purification of BlCMV isolates. The main difference in the two methods was the addition of carbon tetrachloride (CCl_4) and chloroform in Method III and a lower concentration of polyethylene glycol in Method III (5%) than in Method II (6%). The virions of most CABMV isolates were disintegrated by CCl_4, whereas most BlCMV isolates (e.g. BlCMV-Ga isolate) were stable in the presence of CCl_4.

Most of the isolates of BlCMV and CABMV reached maximum concentration in cowpea within 2-3 weeks after inoculation, with higher virus yield than could be recovered from tissue harvested > 3 weeks post-inoculation or from plants resprouted after first leaf harvest.

Production of antiserum:

Antiserum to a typical isolate of CABMV (RN-7C) was produced and standardized for DAC and DAS ELISA tests. The antiserum reacted positively with homologous antigen (RN-7C) and specifically to CABMV isolates (Table 3.7). No reaction to healthy sap occurred. Individual bleeding of antisera to CABMV isolates reacted equally to homologous and heterologous antigens. This antiserum did not react to BlCMV isolates when tested either in DAS or DAC ELISA tests (such data has been shown in Chapter-7).

Standardization of ELISA tests:

The results of ELISA standardization with crude anti-BlCMV (provided by Dr. C.W. Kuhn) and of CABMV (produced in our laboratory, against isolate RN-7C) with blocking and cross absorbance are shown in Table 3.7 & 3.8. The antiserum of CABMV with cross-absorbance in healthy sap at 1000 fold dilution produced about the same A_{405} values as it produced at 500-fold dilution in DAC-ELISA with blocking. Similarly BlCMV antiserum also reacted with homologous antigens at 1000-fold dilution with enough absorbance within 60 min to detect a virus. DAC-ELISA with cross-absorbance was preferred over DAC-ELISA with blocking due to reduction in time and economical use of antiserum.

For DAS-ELISA the IgGs to both BlCMV and CABMV produced absorbance values with extracts from infected tissues significantly different from values produced by healthy extracts at 1000-fold dilutions of IgG and IgG-conjugate against 100-fold dilutions of antigen to distinguish from healthy sap at the same dilution (Table 3.9).

Electron microscopy:

Flexuous rod-shaped particles were consistently found in leaf dip preparations prepared from infected cowpea plants with isolates of BlCMV or CABMV. Occasionally, virus aggregates were also found in purified preparations. The histogram in Figure 2, represents the length of particles

Table 3.7: Results of standardization of crude antiserum of RN-7C (CABMV) for DAC-ELISA with blocking.

Antigen dilutions	A_{405} values after 60 min incubation with substrate.
	Antiserum diluted to 500 fold in VB[a]
A. Purified virus (RN-7C)	
1 ug/ml	0.660
50 ng/ml	0.098
B. Fresh infected tissue (Homologous, 9-7C)	
100 X	1.872
1000 X	0.297
10,000 X	0.052
C. Other CABMV isolates (Heterologous) at 100 X	
RN-4C	1.917
RN-5C	1.414
RN-6C	1.652
D. Healthy sap	
10 X	0.032
100 X	0.013

a: VB, Virus buffer (PBS-Tween + 0.2% egg albumin and 2% polyvinylpyrrolidone PVP 10)

Table 3.8: Results of standardization for DAC-ELISA by cross absorbance, against antisera to CABMV and BlCMV.

Antigen dilutions	A_{405nm} values 60 min after incubation with substrate								
	Antiserum dilutions								
	200			1000			10,000		
	Goat antirabbit IgG-conjugate dilutions								
	200	1000	5000	200	1000	5000	200	1000	5000
Antiserum to CABMV (RN-7C)									
A. Diseased sap (Homologous)									
10 X	>3	2.812	0.615	2.712	1.882	0.151	0.534	0.354	0.170
100 X	>3	1.664	0.450	2.023	1.342	0.245	0.395	0.176	0.176
1000 X	>3	1.862	0.441	2.374	0.883	0.293	0.444	0.247	0.135
B. Healthy sap									
10 X	0.403	0.186	0.157	0.205	0.142	0.120	0.174	0.132	0.142
100 X	0.366	0.196	0.183	0.159	0.122	0.201	0.100	0.130	0.112
Antiserum to BlCMV-Ga									
A. Diseased sap (Homologous)									
10 X	>3	1.486	0.178	2.212	1.901	0.252	0.660	0.562	0.134
100 X	>3	0.952	0.292	>3	1.821	0.262	0.558	0.411	0.139
1000 X	>3	0.997	0.273	2.184	0.810	0.210	0.505	0.209	0.137
B. Healthy sap									
10 X	0.341	0.175	0.133	0.174	0.120	0.194	0.109	0.116	0.125
100 X	0.377	0.137	0.125	0.150	0.115	0.118	0.189	0.187	0.118

Table 3.9: Results of standardization of DAS-ELISA for CABMV and BlCMV virus specific IgG and IgG-conjugate.

Antigen dilutions	A_{405} values after 60 min incubation with substrate								
	IgG dilutions								
	200			1000			10,000		
	IgG-conjugate dilutions								
	200	1000	5000	200	1000	5000	200	1000	5000
CABMV (RN-7C) IgG									
A. Diseased sap (Homologous)									
10 X	>3	1.946	0.533	>3	1.783	0.505	0.510	0.259	0.131
100 X	>3	0.974	0.325	1.855	0.913	0.318	0.131	0.127	0.122
1000 X	>3	0.422	0.191	0.730	0.412	0.206	0.113	0.121	0.111
B. Healthy sap									
10 X	0.300	0.210	0.200	0.165	0.113	0.101	0.106	0.108	0.100
100 X	0.240	0.106	0.175	0.110	0.023	0.011	0.100	0.115	0.103
BlCMV IgG									
A. Diseased sap (Homologous)									
10 X	2.226	2.401	0.853	>3	>3	0.998	1.858	1.233	0.268
100 X	2.410	1.836	0.558	>3	2.336	0.881	1.731	1.178	0.277
1000 X	1.320	0.983	0.997	>3	1.779	1.528	0.552	0.823	0.136
B. Healthy sap									
10 X	0.415	0.315	0.175	0.209	0.163	0.047	0.267	0.081	0.001
100 X	0.327	0.217	0.129	0.217	0.118	0.024	0.184	0.068	0.111

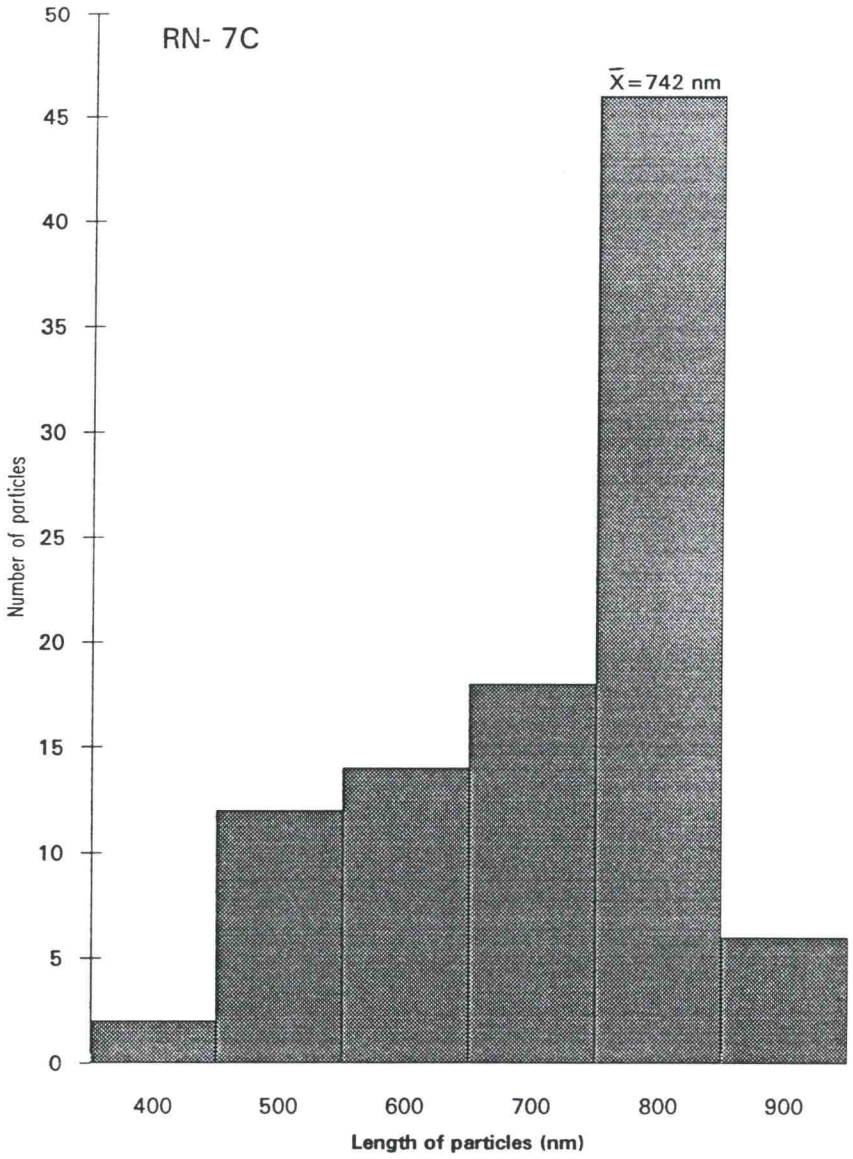


Figure 2. Histogram of particle lengths of CABMV isolate RN-7C. The modal length of virus particles was 742 nm.

distribution of CABMV isolate RN-7C. The figure shows a maximum between 700 to 800 nm for this isolate. The modal length (i.e. the value most frequently occurring) was found to be 742 nm.

DISCUSSION

The choice of a host plant for virus propagation is often critical, and it varies with different virus and virus strains. The tobacco species *N. benthamiana* which gave high yield of RN-7C (CABMV) was not suitable for Georgia isolate of BlCMV, when purified from the same host under same conditions. Some isolates of BlCMV (i.e. BlCMV-Ga, RF-4B, RF-26B) and CABMV (RN-18C, PI-39C) produced very severe symptoms with leaf malformation on *N. benthamiana*, and was difficult to get sufficient leaf tissue for purification. Suitability of tobacco species for virus purification and particularly for potyviruses has been reported (Hollings and Brunt, 1981; Purcifull and Gonsalves, 1985). Virus concentration in the propagation host is also influenced by both the age of the host and environmental conditions. Different strains of a virus often require different periods of time to reach maximum concentration in systemically infected tissue (Steere, 1959).

The average absorbance A_{260}/A_{280} (nucleic acid/protein, absorbing regions) ratios for the purified isolates of BlCMV and CABMV (Table 3.6) were 1.15 and 1.10 respectively,

which are somewhat below reported values of 1.2 for BlCMV and CABMV (Lima et al., 1979) and other potyviruses (Hollings and Brunt, 1981). Slight variation in reported values for individual virus and virus isolates are possible and due to some, but not others, being corrected for light-scattering.

Aggregation of virus particle, and virus and host components during purification have been identified as limiting factors in obtaining higher yields especially in potyviruses (Barnett and Alper, 1977; Hiebert and MacDonald, 1973; Shepherd and Pound, 1960, van Oosten, 1972, Hollings and Brunt, 1981). However, losses associated with low speed centrifugation due to aggregation of virus particles, were reduced by the addition of EDTA and Triton X-100 to grinding and subsequent resuspension buffer. The effectiveness of EDTA and Triton X-100 in reducing virus aggregation during virus purification has been reported for potyviruses (Taiwo et al., 1982; Hollings and Brunt, 1981).

Different potyviruses show widely different tolerance to various clarifying reagents. The most widely used clarifying reagent for potyviruses has been chloroform (Hollings and Brunt, 1981). Carbon tetrachloride has also been used at a concentration of 12.5 - 25% to clarify bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), plumb pox virus, and BlCMV potyviruses (Hollings and Brunt, 1981; Zhao et al., 1991). The purification protocol was improved for

BlCMV isolates and the virus was stable when CCl_4 was used in combination with chloroform in method III, but it did not work for CABMV isolates and disintegration of virus particles was observed. The use of Ccl_4 in combination with chloroform to purify BlCMV (Zaho et al., 1991b) and BYMV, bearded iris mosaic, dasheen onion and yellow dwarf potyviruses (Hollings and Brunt, 1981) has been reported with good results, and we also obtained the same results with BlCMV isolates. In this study we found that borate buffer was better than phosphate buffer for the purification of BlCMV and CABMV isolates. The superiority of borate buffer over phosphate has previously been reported by Shepherd and Pound (1960) with turnip mosaic virus, and by Tomlinson (1964) with lettuce mosaic virus.

Numerous variations of ELISA have been reported for detecting plant viruses (Koening and Paul, 1982; Converse and Martin, 1990), but DAC and DAS ELISA are most commonly used. DAS-ELISA is more specific than DAC-ELISA which allows differentiation of closely related strains (Koening, 1978; Van Regenmortel and Burckhard, 1980) DAC-ELISA detects a broader range of serologically related viruses and these assays are often more sensitive than DAS-ELISA (Van Regenmortel and Buckhard, 1980; Torrance, 1980; Barbara and Clark, 1982). Before conducting any ELISA test, the standardization of antiserum or IgG, IgG-conjugate is essential to determine the optimum levels of reagents to

increase sensitivity and to have reliable results. In this study we standardized the methods which worked well both for DAC and DAS ELISA to detect BlCMV or CABMV. The sodium sulphate precipitation method of purifying immunogamm-aglobulin and the method adopted for IgG-conjugate also worked well with greater sensitivity for detecting these viruses. DAC-ELISA was found to be capable of detecting 1 ug/ ml of purified virus, while DAS-ELISA was capable to detect antigen (fresh virus-infected tissue) when used at 1000-fold dilution.

The modal length of CABMV (RN-7C) virions was found to be 742 nm, which is in agreement with the published results reported for CABMV (Bock, 1973; Lovisolo and Conti, 1974; Edwardson and Christie, 1986; Mali et al., 1988).

CHAPTER-4

BIOLOGICAL CHARACTERIZATION OF SEED-BORNE ISOLATES OF COWPEA APHID-BORNE MOSAIC POTYVIRUS

ABSTRACT

Twenty-one of fifty seed-borne isolates of CABMV derived from U.S. *Vigna* pre-introductions and seed-lots from Pakistan were compared for their biological properties on 51 cowpea genotypes. Selected isolates were also compared on 11 genotypes of bean and 7 of pea, and of local lesion hosts, *Chenopodium amaranticolor, C. album* and *Ocimum basilicum*. CABMV isolates varied significantly in their pathogenicity to 51 cowpea genotypes and two types of local lesion hosts. Host reactions depended on both cowpea genotypes and CABMV isolates, and included immune, resistant, tolerant, and susceptible. Some isolates also induced a hypersensitive response associated with localized lesions or necrosis without systemic infection. However, a few isolates induced localized hypersensitivity followed by systemic mild mosaic or necrotic lethal susceptibility.

Based on differential reactions in 35 cowpea genotypes, 23 distinct variants of CABMV with narrow to wide range were identified. Some variants e.g. PI-44C, PK-16C, and RN-14C induced mild symptoms, whereas the others like CABMV-Mor, PI-39C, and RN-18C were more

virulent. A set of 35 cowpea genotypes distinguishing CABMV variants was established. Sources of *Vigna* resistance to individual as well as to all variants have been identified. Two TVU lines, TVU 401 and TVU 1582, were immune to all CABMV isolates. Purple Hull Pinkeye-BVR was immune to all isolates except CABMV-Mor. Similarly White Acre-BVR and Serido were immune to all CABMV isolates except CABMV-Mor and PI-39C.

It is proposed that virus-resistance breeding programs using identified resistance sources should include testing of hybrid progeny with virulent variants to develop cultivars possessing resistance against the natural variants of CABMV.

INTRODUCTION

In the reproduction of all living organism there is a tendency for progenies to differ from the parents. It is the differential selection of new types or variants that only probably leads to evolution. Viruses are known to have the same capacity to produce variants. Apart from its importance in viral evolution, the occurrence of variants is of great practical importance in agriculture. Strains may differ in the severity of the disease they cause in the field on host cultivars. Breeding programs developing virus-resistant cultivars should include studies on

pathogenic variation among virus isolates; otherwise new cultivars developed for resistance to one viral strain is likely to become susceptible to another.

In this chapter we have discussed the biological properties (local lesions and systemic infection) of 23 seed-borne isolates of CABMV. The specific objectives of the following study were (a) to determine the variability among seed-borne isolates of CABMV in local lesion production on indicator hosts (b) to determine the variants of virus using cowpea genotypes or legume hosts (c) to develop a standard set of cowpea genotypes to be used to differentiate among virus variants, and (d) identification of resistant sources to be included in breeding programs for development of resistant cultivars.

We have used a few terms to describe the types of host response to inoculation with virus isolates, and therefore, it is advisable to define these terms at this stage.

Variant: Any viral isolate with distinctive host relationship is called a variant.

Strain: Strain is a naturally occurring variant of a virus which differs from another by a characteristic response to a particular host genotype.

Pathotype: Pathotype is defined as an entity of a given virus that is controlled by a specific genetic factor in a given plant species or family.

Immune: The host's ability to prevent virus synthesis and disease, i.e., virus is not detectable by ELISA after inoculation. Opposite of susceptibility.

Resistant: The ability of a host plant to suppress or retard viral multiplication with little symptom development. There are degrees of resistance e.g. extreme, moderate, or low level of resistance.

Tolerance: Host response to viral infection resulting in negligible or mild disease development, with readily detectable levels of virus concentration within the host.

Hypersensitivity: It is the host response by which virus infection is localized to a zone of cells around the initial infection cells site, usually with the formation of visible chlorotic or necrotic local lesions and no systemic spread.

Lethal susceptible: The host response against virus inoculation in which the whole plant necrosis results with complete killing of plants within two to four weeks of inoculation.

MATERIALS AND METHODS

Virus isolates:

Twenty-one out of 50 isolates of CABMV derived from naturally infected U.S. *Vigna* pre-introductions and seed lots from Pakistan, were selected for the study of their biological properties on cowpea (*Vigna unguiculata*), bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), and selected local lesions hosts. The origin and other details of isolates included in this study are presented in Appendix-2. A culture of each isolate was maintained in susceptible cowpea genotypes (PI 218123, PI 251222 or Pusa Phalguni) by infrequent mechanical transmission under insect-free greenhouse conditions. Precautions were adopted as described in Chapter-2 to minimize isolate contamination. The Morocco isolate of CABMV, a tentative type isolate of the virus, was kindly provided by Dr.D. Gonsalves, Department of Plant Pathology, Cornell University, Geneva, NY 14456, U.S.A., and included for comparison. A Georgia isolate of BlCMV obtained from Dr. C.W. Kuhn served as the reference isolate for BlCMV.

Source of cowpea genotypes:

Fifty-one cowpea genotypes (Table 4.2) were obtained from the following locations: six TVU lines (Tropical *Vigna unguiculata*) from International Institute of Agriculture (IITA), Ibadan, Nigeria, 22 promising U.S.

cowpea cultivars from Dr.O.L. Chambliss, Auburn University, Department of Horticulture, Alabama 36849, U.S.A., and 23 advanced and TVU lines from Drs. A.E. Hall and P.N. Patel, University of California, Riverside, California, U.S.A. Seeds of most cultivars/lines were increased in insect-free greenhouse conditions to ensure freedom from seed-borne diseases and to produce sufficient seed supplies. Seeds of bean, pea and local lesion hosts were available in the Virology Laboratory, Department of Botany and Plant Pathology, Oregon State University, Corvallis OR U.S.A.

Screening of cowpea genotypes for resistance:

Ten to 12 seeds of each cowpea genotype were grown in plastic pots filled with sterilized soil. Seven to eight days after planting and when the primary leaves were fully expanded, seedlings were inoculated by standard procedure as described by Noordam (1973). Leaves of plants infected with each isolate were homogenized in buffer (0.02 M phosphate buffer pH 7.0) and applied as inoculum to carborundum-dusted leaves. The same number of plants of the most susceptible genotype (PI 218123 or PI 251222) were simultaneously inoculated with the same inoculum, to monitor inoculation effectiveness. Two weeks after inoculation, at symptom induction, symptomless plants of each treatment were reinoculated to minimize escapes. Non-inoculated, buffer-abraded plants served as negative controls. Inoculated test plants were kept under

observation in the greenhouse for five weeks at temperatures of 28-30 C. Inoculated primary leaves were observed for localized reactions and whole plants for systemic symptoms, recorded at weekly intervals. Composited leaf-tissue samples from symptomless plants of each treatment were tested by DAS-ELISA to distinguish immunity from symptomless infection (tolerance). The experiment was terminated five weeks after first inoculations.

Tests with bean and pea genotypes:

Eleven genotypes of bean and seven of pea were tested against selected seed-borne CABMV isolates, to determine their biological variability on these alternate legume hosts. Screening procedures were similar to those described for screening of cowpea genotypes. DAS-ELISA tests were also performed on symptomless plants that had been inoculated with each isolate. Observations on local and systemic reactions were recorded at weekly intervals, until 4 weeks after inoculation.

Local lesion tests:

Three local lesion species, *Chenopodium amaranticolor*, *C. album* and *Ocimum basilicum* (basil) were inoculated with 21 CABMV seed-borne isolates to assess their differential reactions. Four leaves of young plants were mechanically inoculated with each isolate essentially as described by Noordam (1973). Leaves were examined for local lesion development for 3 weeks after inoculation.

Because some plants did not develop local lesions, selected genotypes were assayed for systemic infection by DAS-ELISA three weeks after inoculation. Plants were grown under standard greenhouse conditions. Numbers of local lesions induced by each isolate were recorded at weekly intervals.

RESULTS

Symptomatology and genotypic response:

Inoculations with each isolate produced a wide range of disease symptoms. The responses of 51 cowpea genotypes to inoculations with 21 CABMV seed-borne isolates ranged from rapid development of whole plant necrosis to immunity Very characteristic symptoms were induced on the standard susceptible cowpea genotypes by most of the isolates. Induced symptoms varied with virus isolate-cowpea combination. Diseased cowpea plants typically developed variable degrees of chlorotic vein-banding, interveinal chlorosis, leaf distortion, "blistering", mild to severe mosaic and stunting (Figure 3 and 4). Reactions of inoculated leaves included either necrotic or chlorotic local lesions (hypersensitivity); no systemic infection or the absence of symptoms, the latter being more common response. In some cases, inoculated leaves showed yellowing and premature defoliation, irrespective of subsequent plant response. A few isolates induced necrotic lesions on

Figure 3. CABMV symptoms under field and greenhouse conditions.

(a). Symptoms of seed-borne CABMV in seedlings from virus-infected seeds, grown under field conditions.

(b). Symptoms of seed-borne CABMV isolate RN-27C in the '58-57' genotype from which the isolate was originally recovered. Seeds were germinated under greenhouse conditions.

Figure 4. Severe mosaic and necrotic lesions induced by CABMV isolate PI-39C.

(a). Severe mosaic in cowpea genotype PI 251222, induced by mechanically inoculation with CABMV isolate PI-39C under greenhouse conditions.

(b). Necrotic lesions and systemic bud necrosis induced in cowpea genotype TVU 408P2, by CABMV isolate PI-39C.

inoculated primary leaves followed by whole plant necrosis (lethal susceptible) (Figure 5). In general four types of isolate-genotype interactions, immune, resistant, tolerant and susceptible were obtained (Table 4.1). Some genotypes showed delayed symptoms (DS), the plants of these lines were generally free of symptoms for 2-3 weeks, but subsequently developed mild to severe mosaic symptoms depending on the genotypes. Such genotypes might easily have been rated as immune or resistant if observations had not been extended to 5 weeks post-inoculation. Disease reactions of each isolate against each genotype are presented in Table 4.2.

Identification of resistant/susceptible genotypes:

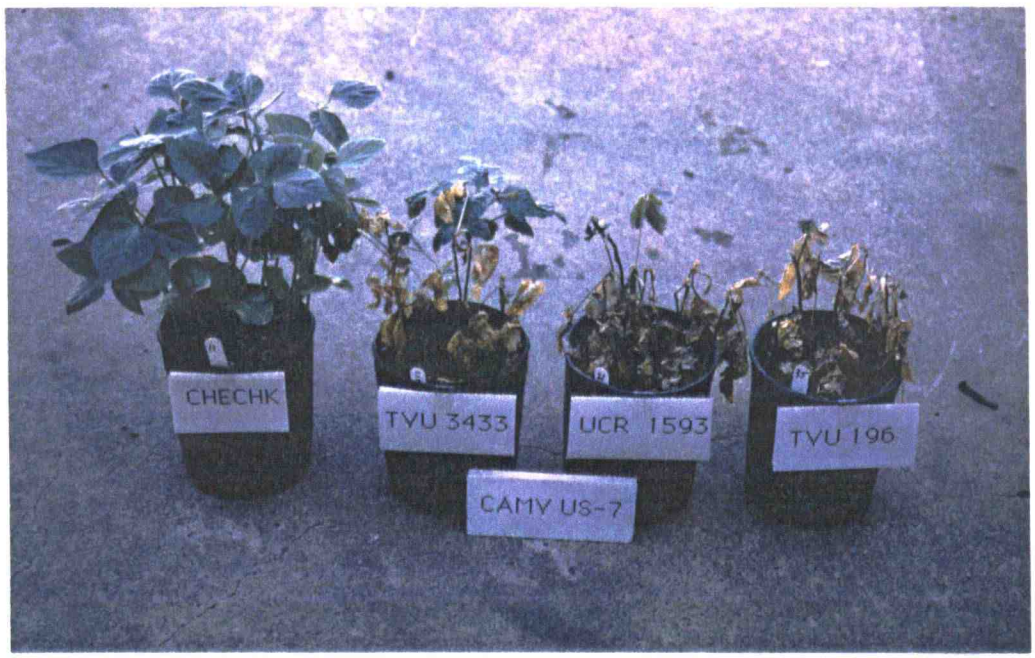
Two types of symptomless responses were defined: immunity and tolerance, immunity being more common. Two TVU lines, TVU 401 and TVU 1582 were immune to all 50 CABMV seed-borne isolates having geographical diverse origins. Several genotypes that were immune, resistant or tolerant to individual isolates were also identified (Table 4.3).

Differential reactions and pathogenic variation among virus isolates:

Eight of 51 genotypes were susceptible to all isolates (Table 4.3). Thirty-five (68%) genotypes produced differential reactions to 23 isolates ranging from immunity, tolerance to susceptibility (Table 4.4). Genotype PI 218123, which was susceptible to all 50 isolates,

Figure 5. Lethal susceptibility of cowpea genotypes against CABMV isolates.

(a). Lethal susceptibility of cowpea genotype PI 218123 when inoculated with CABMV-Mor, shown two weeks after inoculation.



(b). Lethal susceptibility of cowpea genotypes when inoculated with CABMV isolate US-1C, shown four weeks after inoculation.

Table 4.1: Responses of cowpea genotypes to mechanical inoculation with CABMV isolates.

Reaction types	Description
Immune:	No visible symptoms, no ELISA detectable virus.
Resistant:	a. Localized Hypersensitivity; localized lesions, no systemic infection. b. Asymptomatic infection, low virus concentration.
Tolerant:	Latent infection, with or without primary lesions, virus detectable by ELISA.
Susceptible:	a. Moderate to severe systemic mosaic. b. Local lesions, followed by whole plant necrosis (lethal susceptible)

Table 4.2: Disease reactions of cowpea genotypes against CABMV isolates 4 weeks after mechanical inoculation.

Genotype	Virus isolates													
	Mor		Bz		RN-4C		RN-6C		RN-7C		RN-10C		RN-11C	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	1		2		3		4		5		6		7	
TVU 109P2	.[a]	+++	.	+++	.	-	.	-	.	-	.	-	.	-
TVU 196	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 347	.	+++	.	+++	.	-	.	-	.	-	.	-	NL,V	Li
TVU 354	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 401	.	-	.	-	.	-	.	-	.	-	.	-	.	-
TVU 408P2	.	-	NL,V	Ls	NL,V	++	NL,V	+	NL	-	.	-	.	-
TVU 410	.	-	NL,V	Ls	NL,V	++	NL,V	+	NL	-	.	-	NL,V	Li
TVU 984	.	+++	.	+++	.	++	.	+++	.	+++	.	-	NL,V	+
TVU 1000	.	+++	.	-	.	-	.	-	.	-	.	-	.	+++
TVU 1016-1	.	+++	.	-	.	-	.	-	.	-	.	-	NL,V	Ls
TVU 1582	.	-	.	-	.	-	.	-	.	-	.	-	.	-
TVU 1593	.	-	.	-	.	-	.	-	.	-	.	-	NL,V	-
TVU 2657	.	+++	.	+++	.	-	.	+++	.	+++	.	+++	.	+++
TVU 2740	.	+++	.	+++	.	-	.	+++	.	+++	.	Li	.	+++
TVU 2845	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 3433	.	+++	.	+++	.	-	.	+++	.	+++	.	Li	.	+++
IT 80S 2049	.	+++	o	o	.	+++	.	+++	.	+++	.	+++	.	+++
CBE 3	.	+++	.	-	.	-	.	-	.	Li	.	-	.	-
CBE 5	.	+++	.	+++	.	+++	.	+++	NL	++	.	++	.	+++
CBE 46	.	+++	.	+++	.	+++	.	+++	NL	Li	.	-	.	-
CBE 88	.	+++	.	+++	.	++	.	+++	NL	++	.	-	.	+++

Cont,d..

Table 4.2: (Cont,d..)

Genotype	Virus isolates													
	Mor		Bz		RN-4C		RN-6C		RN-7C		RN-10		RN-11C	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	1		2		3		4		5		6		7	
UCR 7964	.	+++	.	+++	.	+++	.	+++	NL	++	.	-	.	+
UCR 8517	.	-	.	+++	.	+++	.	+++	NL	-	.	-	.	+++
UCR 8679	.	+++	.	+++	.	-	.	-	.	++	.	-	.	-
UCR 160	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
UCR 1393	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
UCR 524B	.	+++	.	+++	.	+++	NL	+++	.	+++	.	++	.	+++
P.P.H.BVR	.	+++	.	-	.	-	.	-	.	-	.	-	.	-
White.BVR	.	+++	.	-	.	-	.	-	.	-	.	-	.	-
Serido	.	+++	.	-	.	-	.	-	.	-	.	-	NL,V	-
Bettergreen	.	+++	.	-	.	-	.	-	.	-	.	-	NL,V	-
M. Purple	.	+++	.	+++	.	Li	.	Li	.	+++	.	Li	NL,N	++
M. Silver	.	+++	.	+++	o	o	o	o	.	Li	o	o	NL,V	++
Mopod	.	+++	.	-	.	++	.	-	.	-	o	o	o	o
Corona	.	+++	.	-	.	-	.	-	.	-	o	o	.	-
Worthmore	.	+++	.	+++	.	Li	.	Li	.	+++	o	o	NL,V	-
T.W.Crowder	.	+++	.	+++	.	-	.	-	.	+++	o	o	.	-
T.Cream # 8	.	+++	.	-	o	o	.	-	.	-	o	o	.	++
T.Cream # 40	.	+++	.	-	.	-	NL	-	NL	-	o	o	.	-
Blue Goose	.	+++	.	-	.	-	.	-	.	++	o	o	o	o
M.Shipper	.	Li	.	+++	.	Li	NL,V	Li	.	++	o	o	NL	+++
Snappea	.	+++	o	o	o	o	o	o	.	++	o	o	o	o

Cont,d..

Table 4.2: (Cont,d..)

Genotype	Virus isolates													
	Mor		Bz		RN-4C		RN-6C		RN-7C		RN-10		RN-11C	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	1		2		3		4		5		6		7	
Big Boy	.	+++	.	-	.	-	.	-	.	-	o	o	.	-
B.S.Crowder	.	+++	.	+++	.	-	.	++	.	+++	.	+++	NL	+++
M. Cream	.	+++	.	+++	.	-	.	-	.	+++	o	o	.	Li
Magnolia	.	+++	.	+++	.	Li	NL	-	NL	+++	o	o	NL	+++
K.P. Hull	.	+++	.	-	.	Li	NL	-	.	-	.	-	.	-
T. Pinkeye	.	+++	.	-	.	-	NL	-	.	-	.	-	.	-
Pusa Phalguni	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
PI 218123	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
PI 251222	NL,V	Ls	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++

[a]**Symbols used for virus-induced symptoms in Table 1.** Cont,d..

. = No hypersensitive response (no local lesion production); o = Not tested;
- = Immune, no visible symptoms and no virus detection by ELISA;
+ = Very mild systemic mosaic; ++ = Moderate type systemic mosaic, not much severe; +++ = Very clear characteristic virus symptoms (highly susceptible);
Li = Latent infection, no visible symptoms, virus detected only by ELISA; NL = Necrotic local lesion (hypersensitive response); V = Vein necrosis on the inoculated primary leaves. Ls = Lethal susceptible, the plants were killled completely within two week after inoculation; Lo = Local reaction;
Sy = Systemic reaction

Table 4.2: (Cont,d..)

Genotype	Virus isolates													
	RN-12C		RN-18C		PI-23C		RN-27C		RN-28C		RN-29C		RN-34C	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	8		9		10		11		12		13		14	
TVU 109P2	.	-	.	-	.	++	.	-	.	-	.	-	.	-
TVU 196	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 347	.	-	.	-	.	+++	.	-	.	-	.	-	.	-
TVU 354	.	+++	.	+++	.	+++	.	+++	.	-	.	+++	.	+++
TVU 401	.	-	.	-	.	-	.	-	.	-	.	-	.	-
TVU 408P2	NL,V	-	NL,V	++	NL,V	+	NL	-	NL	-	NL,V	-	.	-
TVU 410	NL,V	++	NL,V	++	.	++	NL	-	NL	-	NL,V	++	.	-
TVU 984	.	+++	.	+++	.	+++	.	-	.	-		+++	.	++
TVU 1000	.	-	.	-	.	+++	.	-	.	-	.	-	.	-
TVU 1016-1	.	-	.	-	.	-	.	-	.	-	.	-	.	-
TVU 1582	.	-	.	-	.	-	.	-	.	-	.	-	.	-
TVU 1593	.	-	.	-	.	-	.	Li	.	-	.	+++	.	-
TVU 2657	.	-	.	+++	.	-	.	-	.	+++	.	+++	.	+++
TVU 2740	.	Li	.	+++	.	Li	.	-	.	+++	.	+++	.	-
TVU 2845	.	+++	.	o	.	+++	.	-	.	+++	NL,V	+++	NL,V	+++
TVU 3433	.	Li	.	+++	.	Li	.	Li	NL,V	+++	.	+++	.	-
IT 80S 2049	.	+++	NL	+++	NL	++	NL,V	-	.	+++	o	o	NL	+
CBE 3	.	-	.	++	.	-	.	-	.	-	.	-	.	Li
CBE 5	.	++	.	+++	.	++	.	+++	.	Li	.	+++	.	+++
CBE 46	.	+++	.	+++	.	L	.	+++	.	Li	.	+++	.	+++
CBE 88	.	+++	.	+++	.	Li	.	++	.	Li	.	++	.	+++

Cont,d..

Table 4.2: (Cont,d..)

Genotype	Virus isolates													
	RN-12		RN-18C		PI-23C		RN-27C		RN-28C		RN-29C		RN-34C	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	8		9		10		11		12		13		14	
UCR 7964	.	+++	.	+++	.	-	.	+++	.	++	.	-	.	+ + +
8517	.	++	.	+++	.	Li	.	++	.	-	.	-	.	++
UCR 8679	.	++	.	++	.	-	.	-	.	-	.	-	.	-
UCR 160	.	+++	.	++	.	++	.	+++	.	++	.	+++	.	+++
UCR 1393	.	+++	.	+++	.	+	.	+++	.	++	.	+++	.	+++
UCR 524B	.	+++	.	+++	.	++	NL	+++	.	++	.	+++	.	+++
P.P.H.BVR	.	-	.	-	.	-	.	-	.	-	.	-	.	-
White.A.BVR		-	.	-	.	.	.	-	.	-	.	-	.	-
Serido	.	-	.	-	.	-	.	-	.	-	.	-	.	-
Bettergreen	.	-	.	-	.	-	.	-	.	-	o	o	.	-
M. Purple	.	+++	.	+++	.	-	.	-	NL,V	+++	.	+++	.	++
M. Silver	.	+++	.	+++	.	-	.	++	NL,V	+++	o	+++	o	o
Mopod	o	o	.	+++	.	-	o	o	o	o	o	+++	.	+++
Corona	.	-	.	-	.	o	o	o	.	-	o	++	o	o
Worthmore	.	+++	.	+++	.	Li	.	-	NL,V	+++	o	+++	.	+++
T.W.Crowder	.	+++	.	+++	.	-	.	-	.	Li	o	+++	o	o
T.Cream # 8	.	-	.	-	.	-	.	Li		++	o	Li	o	o
T.Cream # 40	.	-	.	-	.	-	NL	Li	.	-	o	-	o	o
Blue Goose	.	-	.	-	.	-	.	-	.	Li	o	o	o	o
M.Shipper	o	o	.	+++	.	-	.	-	.	+++	o	+++	.	+++
Snappea	o	o	.	+++	.	o	o	-	o	o	o	o	o	o

Cont,d..

Table 4.2: (Cont,d..)

Genotype	Virus isolates													
	RN-12		RN-18C		PI-23C		RN-27C		RN-28C		RN-29C		RN-34C	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	8		9		10		11		12		13		14	
Big Boy	.	-	.	+++	.	-	.	-	.	-	o	-	o	o
B.S.Crowder	.	+++	.	+++	.	Li	.	+++	.	+++	.	+++	.	+++
M. Cream	o	o	.	+++	o	o	.	-	.	+++	o	+++	.	+++
Magnolia	.	+++	.	+++	.	Li	NL	+++	NL,V	+++	.	+++	.	+++
K.P. Hull	.	-	.	-	.	Li	NL	++	NL,V	Li	.	-	.	-
T. Pinkeye	.	-	.	-	.	-	NL	++	.	Li	o	o	.	Li
Pusa Phalguni	NL	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
PI 218123	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
PI 251222	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++

Cont,d..

Table 4.2: (Cont,d..)

Genotype	Virus isolates													
	RN-35C		PI-39C		PI-40C		PI-41C		PI-42C		PI-43C		RN-44C	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	15		16		17		18		19		20		21	
TVU 109P2	.	-	.	+++	.	Li	.	+++	.	Li	.	+++	.	Li
TVU 196	.	+++	.	+++	.	+++	.	Li	.	Li	.	+++	.	-
TVU 347	.	-	.	+++	.	Li	.	+++	.	Li	.	+++	.	-
TVU 354	.	++	.	+++	.	+++	.	+++	.	Li	.	+++	.	-
TVU 401	.	-	NL,V	Ls	.	-	NL	-	.	-	.	-	.	-
TVU 408P2	.	-	NL,V	+	NL,V	+	.	-	NL	-	.	-	.	-
TVU 410	.	-	NL,V	+	Nl,V	+	.	-	NL	-	.	-	.	-
TVU 984	.	-	.	+++	.	+++	.	++	.	Li	.	+++	.	-
TVU 1000	.	-	.	+++	.	-	.	+++	.	+++	.	+++	.	-
TVU 1016-1	.	-	.	+++	.	-	NL	+++	.	++	.	+++	.	Li
TVU 1582	.	-	NL,V	Ls	.	-	.	-	.	-	.	-	.	-
TVU 1593	.	-	NL,V	Ls	.	Li	.	+++	.	+++	.	-	.	-
TVU 2657	.	++	NL.V	Ls	.	Li	.	++	.	++	.	+++	.	-
TVU 2740	.	++	NL,V	Ls	.	++	.	+++	.	-	.	++	.	-
TVU 2845	.	+++	.	+++	o	o	o	o	o	o	.	-	NL,V	-
TVU 3433	.	+++	.	Ls	.	+++	.	+++	-	-	.	-	.	-
IT 80S 2049	o	o	NL	+++	.	-	.	-	.	-	.	-	.	-
CBE 3	.	-	.	-	.	+++	.	++	.	-	o	o	.	-
CBE 5	.	+++	.	+++	.	++	.	++	.	+++	o	+++	.	++
CBE 46	.	+++	.	+++	.	++	.	++	.	-	o	o	.	-
CBE 88	.	+++	.	+++	.	++	.	++	.	-	o	o	.	-

Cont,d..

Table 4.2: (Cont,d..)

Genotype	Virus isolates													
	RN-35		PI-39C		PI-40C		PI-41C		PI-42C		PI-43C		PI-44C	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	15		16		17		18		19		20		21	
UCR 7964	.	+++	.	+++	.	++	.	++	.	-	o	o	.	-
UCR 8517	.	+++	.	+++	.	++	.	++	.	-	o	o	.	-
UCR 8679	.	+++	.	+++	.	-	.	-	.	-	o	o	.	-
UCR 160	.	-	.	+++	.	++	.	++	.	++	.	-	.	Li
UCR 1393	.	+++	.	+++	.	+++	.	+++	.	+++	.	++	.	++
UCR 524B	.	+++	.	+++	.	+++	NL	+++	.	++	.	++	.	++
P.P.H.BVR	.	-	.	-	.	-	.	-	.	-	.	-	.	-
White.A.BVR	.	-	.	+++	.	-	.	-	.	-	.	-	.	-
Serido	.	-	.	+++	.	-	.	-	.	-	.	-	.	-
Bettergreen	.	-	o	o	o	o	o	o	.	+++	.	Li	.	Li
M. Purple	.	+++	.	+++	.	-	.	-	.	-	.	-	.	-
M. Silver	.	+++	.	+++	.	+++	o	-	.	-	.	-	.	-
Mopod	.	+++	.	+++	.	-	.	-	o	o	.	o	.	-
Corona	.	-	.	-	.	-	.	-	.	-	.	o	.	o
Worthmore	.	+++	.	+++	.	+++	.	-	.	++	.	-	.	-
T.W.Crowder	.	+++	.	+++	.	+++	.	-	.	-	.	Li	.	-
T.Cream # 8	.	Li	.	+++	.	-	.	-	.	-	o	o	.	-
T.Cream # 40	.	Li	.	-	.	-	.	+++	-	-	.	-	.	
Blue Goose	.	-	.	-	.	-	.	-	.	-	.	-	.	-
M.Shipper	.	+++	.	+++	.	+++	.	-	.	-	.	-	.	-
Snappea	o	o	.	-	.	-++	.	-	o	o	.	-	.	-
Big Boy	.	-	.	-	.	-	.	-	.	-	.	-	.	-

Cont,d..

Table 4.2: (Cont,d..)

Genotype	Virus isolates													
	RN-35		PI-39C		PI-40C		PI-41C		PI-42C		PI-43C		PI-44C	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	15		16		17		18		19		20		21	
B.S.Crowder	.	+++	.	+++	.	+++	.	-	.	-	.	-	.	-
M. Cream	o	o	.	-	.	-	.	-	.	-	.	-	.	-
Magnolia	.	+++	.	+++	.	+++	.	-	.	-	.	-	.	-
K.P. Hull	.	-	.	-	.	-	.	+	.	-	.	-	.	-
T. Pinkeye	.	-	.	+++	o	o	o	o	.	-	.	-	.	-
Pusa Phalguni	NL	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	++
PI 218123	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
PI 251222	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++

Table 4.3: Grouping of cowpea genotypes based on their reactions against CABMV isolates.

Reaction type	Genotypes falling under each group
Immune (2) (To all isolates)	TVU 401, TVU 1582
Immune/resistant/ Tolerant which showed differential reaction. (41)	TVU 109P2, TVU 347, TVU 354, TVU 408P2, TVU 410, TVU 984,TVU 1000, TVU 1016-1, TVU 1593, TVU 2657, TVU 2740, TVU 2845, TVU 3433, CBE 3, CBE 46, CBE 88, UCR 7964, UCR 8517, UCR 8679, White Acre-BVR, Serido, Purple Hull Pinkeye-BVR, Bettergreen, Mississippi Purple, Mississippi Silver, Mopod, Corona, Worthmore, Texas White Crowder, Texas Cream, Texas Cream # 8, Texas Cream #40, Blue Goose, Mississippi Shipper, Brown Sugar Crowder, Mississippi Cream, Knuckle Purple Hull, Texas Pinkeye, IT 80S 2049. Big Boy and Magnolia
Susceptible (8) (To all isolates)	TVU 196, CBE 5, UCR 160, UCR 1393, UCR 524B, Pusa Phalguni, PI 218123, PI 251222.

Table 4.4: Differential reactions of CABMV isolates induced in cowpea genotypes and arranged according to pathogenic variation among isolates.

Cultivar	Host group	CABMV variant groups																							No of variants attacing each Cv.
		I			II																III				
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	V	U	W	
Big Boy	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	2
W.A.BVR		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	2
Serido		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	2
T.C.#40		-	-	-	-	-	t	-	-	-	-	-	-	-	-	-	t	-	-	-	-	-	-	+	3
CBE 3		-	-	-	-	-	-	-	-	-	-	-	-	t	-	-	-	-	t	-	-	+	-	+	4
B.Goose		-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	t	+	-	-	-	-	+	4
T.Pinkeye		-	-	-	-	-	+	-	-	-	-	-	-	t	-	-	-	t	-	-	-	-	+	+	4
B.green		-	t	-	+	-	-	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	5
K.P.Hull		-	-	-	-	-	+	-	t	-	-	-	-	-	t	t	-	t	-	-	-	-	-	+	6
TVU 1016		t	t	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	7
TVU 1593		-	-	+	+	-	t	+	-	+	-	-	+	-	-	-	-	-	-	-	t	-	-	-	7
UCR 8679		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	7
T.C. #8		-	-	-	-	-	t	-	-	-	+	-	t	-	-	-	t	+	-	-	-	-	+	+	7
TVU 408P2		-	-	+	-	-	-	-	t	-	-	-	-	-	+	t	-	-	-	-	+	+	+	-	7
M.Cream	2	-	-	-	-	-	-	-	-	-	t	-	+	+	-	-	-	+	+	+	-	+	-	+	8
TVU 1000		-	+	-	+	-	-	+	-	+	+	-	-	-	-	t	-	-	-	-	-	-	+	+	8
TVU 410		-	-	-	-	-	-	-	t	-	t	+	+	-	+	t	-	-	-	-	+	+	+	-	9
TVU 109P2		t	t	-	t	-	-	+	-	+	-	-	-	-	-	+	-	-	-	+	t	-	+	+	10
TVU 347		-	t	-	t	-	-	+	-	+	+	-	-	-	-	+	-	-	-	+	t	-	+	+	10
T.W.Crod.		-	-	-	-	+	-	t	-	-	-	+	+	-	-	-	+	t	+	+	+	+	+	+	12
UCR 8517		-	-	-	-	-	+	-	+	+	+	+	-	+	+	t	+	-	-	+	+	+	+	-	13
M.Shipper		-	-	-	-	+	-	-	t	-	+	-	+	+	t	-	+	+	+	+	+	+	+	t	14

Cont,d.

Table 4.4: (Cont,d..)

Cultivar	Host group	BlCMV variant groups																						No of variants attacking
		I			II													III						
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	V	U	W	
TVU 2740	3	-	-	+	-	t	-	-	-	+	+	+	t	-	+	t	+	+	+	+	+	+	+	+	15
M.Purple		-	-	-	-	t	-	-	t	-	+	+	+	+	t	-	+	+	+	+	+	+	+	+	15
B.S.Crow.		-	-	-	-	-	-	-	-	-	+	+	+	+	-	t	+	+	+	+	+	+	+	+	15
IT80S2049		-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	15
TVU 984		-	-	+	t	-	-	-	-	+	-	+	+	+	+	+	-	1	+	+	+	+	+	+	16
TVU 2657		-	-	+	-	-	-	-	-	+	+	-	+	+	+	-	+	+	+	+	t	+	+	+	16
TVU 3433		-	-	+	-	t	t	-	-	+	+	t	+	-	+	t	+	+	+	+	+	+	+	+	16
UCR 7964		-	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+	+	+	+	1	+	16
Worthmore		-	-	-	-	-	-	-	t	-	-	+	+	+	t	t	+	-	+	+	+	+	+	+	16
Magnolia		-	-	-	-	-	-	-	t	-	+	+	+	+	t	t	+	-	+	+	+	+	+	+	16
CBE 46		-	-	-	-	-	-	-	-	+	-	+	+	+	+	t	+	t	t	+	+	+	+	+	17
CBE 88		-	-	-	-	-	-	-	-	+	+	+	+	+	+	t	+	t	+	+	+	+	+	+	18
TVU 354		-	t	+	t	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	21
		2	6	7	10-11		13-14			15-17							18-19				21-31				

- : Immune
A : PI-44C
B : PK-16C
C : RN-14C
D : PI-42C
E : RN-10C
F : RN-27C
G : PI-43C

t : Tolerent
H : RN-4C
I : PI-41C
J : RN-11C
K : RN-12C
L : RN-29C
M : RN-34C
N : RN-6C

O : PI-23C
P : RN-35C
Q : RN-28C
R : RN-7C
S : CABMV-Bz
T : PI-40C
U : RN-18C

+ : Susceptible
V : PI-39C
W : CABMV-Mor

induced necrotic lesions with subsequent lethal susceptibility when inoculated with the CABMV-Mor isolate, distinct from the other 49 CABMV isolates.

Thirty-five out of 51 cowpea genotypes produced differential reactions against 23 CABMV isolates (Table 4.4). Differential genotypes were arrayed into three groups based on the number of isolates attacking each genotype. Differential Groups 1, 2 and 3 included genotypes which are attacked by 2-7, 8-14, and 15-21 isolates respectively. Virus isolates were also arrayed into three groups based on their range of pathogenicity (number of genotypes attacked by each isolate). Groups 1, 2 and 3 included the isolates attacking 2-11, 13-19, 21-32 genotypes, respectively.

Response of bean and pea genotypes:

Responses of bean and pea genotypes against CABMV isolates are shown in Table 4.5. All genotypes of bean were immune to all CABMV isolates except Black Turtle Soup Type 2, which produced necrotic local lesions and vein necrosis on the inoculated primary leaves with mild systemic mosaic. Similarly all pea genotypes were immune to all CABMV isolates except, that the RN-7C isolate asymptomatically infected Sounder, Badger and 15491.

Table 4.5: Responses of bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*) genotypes against CABMV isolates.

Host genotype	Virus isolates							
	4C	7C	10C	23C	28C	39C	Mor	BCMV(TS)
A. Beans								
Red Kidney	-[a]	-	-	-	-	-	-	+++
Hystyle	-	-	-	-	-	-	-	-
Dwarf Hort.	-	-	-	-	-	-	-	+++,st
Great Nort.	-	-	-	-	NL/-	-	-	-
91 G	-	-	-	-	NL/-	-	-	-
Pinto	-	-	-	-	-	-	-	-
B.T.Soup-1	-	-	-	-	0	0	-	-
B.T.Soup-2	NL/L	NL/+	NL/+	NL/+	0	0	NL/+	+++
Bountiful	-	-	-	-	-	-	-	+++
Stringless Green Refugee	-	-	-	-	0	-	-	+++
B. Peas								
Ceras	-	-	-	-	0	-	-	0
Charger	-	-	-	-	0	-	-	0
Dual	-	-	-	-	0	-	-	0
Sounder	-	L	-	-	0	-	-	0
D.G. Suger	-	-	-	-	0	-	-	0
Badger	-	L	-	-	0	-	-	0
15491	-	L	-	-	0	-	-	0

[a] **Symbols used for virus-induced symptoms in Table 4.5.**

- = Immune, no symptoms and no ELISA detectable virus; + = Mild systemic mosaic; +++ = Sever systemic mosaic (susceptible); NL = Necrotic lesions L = Latent infection (ELISA detectable virus); st = Stunting; 0 = Not tested. B.T.Soup = Black Turtle Soup; Hort. =Horticulture.

Local lesion tests:

Nineteen out of 21 CABMV isolates were tested on *C. amaranticolor*, three on *C. album*, and six on *Ocimum basilicum*. CABMV isolates produced variable numbers of local lesions on *C. amaranticolor* except that RN-34C and RN-38C produced no local lesion on this host (Table 4.6). Three isolates, RN-4C, RN-7C and CABMV-Bz produced about equal number of lesions on *C. album*. Local lesions produced by *O. basilicum* took the form of black rings or solid spots 1-4 mm in diameter two weeks after inoculation. Three of the six isolates, RN-35C, RN-37C and CABMV-Mor tested on four types of basil (*Ocimum basilicum*), induced no local lesions on any basil genotypes. Isolates RN-7C and RN-12C induced 23 and 14 local lesions respectively only on basil type 4. CABMV-Bz induced local lesions on basil type 1 and 2. The size of local lesions produced on either of three hosts varied from pin-head to large lesions (1-3 mm) with distinctive isolate-host combinations.

DISCUSSION

Results of the interactions of virus isolate-host genotype combinations clearly indicated that seed-borne CABMV isolates differ greatly in their biological properties. A wide variation in symptoms induced by CABMV isolates was observed in different cowpea genotypes.

Table 4.6: Local lesions induced by CABMV isolates.

Isolate	Host	No. of local lesions induced
RN-2C	*C. amaranticolor*	30[a]
RN-6C	"	28
RN-7C	"	20
RN-10C	"	35
RN-12C	"	50
RN-18C	"	55
PK-1C	"	20
PK-12C	"	42
PK-17C	"	15
PK-18C	"	18
PK-20C	"	22
RN-34C	"	0
RN-37C	"	45
RN-38C	"	0
PI-39C	"	62
PI-42C	"	59
PI-48C	"	60
CABMV-Bz	"	52
CABMV-Mor	"	60
RN-4C	*C. album*	45
RN-7C	"	50
CABMV-Bz	"	49
	Basil (*Ocimun basilicum*)	
RN-7C	" (Tupe-1)	0
	" (Type-2)	0
	" (Type-3)	0
	" (Type-4)	23
RN-12C	Basil (Tupe-1)	0
	" (Type-2)	0
	" (Type-3)	0
	" (Type-4)	14
RN-35C	Basil (Tupe-1)	0
	" (Type-2)	0
	" (Type-3)	0
	" (Type-4)	0

Cont,d..

Table 4.6 (Cont,d..)

Isolate	Host	No. of local lesions induced
RN-37C	Basil (Tupe-1)	0
	" (Type-2)	0
	" (Type-3)	0
	" (Type-4)	0
CABMV-Bz	Basil (Tupe-1)	18
	" (Type-2)	10
	" (Type-3)	0
	" (Type-4)	0
CABMV-Mor	Basil (Tupe-1)	0
	" (Type-2)	0
	" (Type-3)	0
	" (Type-4)	0
		0

[a] Number represents average value of local lesions induced in four leaves of each species.

0 : No local lesion induced.

Characteristic symptoms expressed in certain infected plants are useful in separating viruses and their strains. But symptoms are often unsuitable for identification, because different viruses may induce similar symptoms in the same plant or the same virus may induce very different symptoms in different genotypes.

Variability among isolates of CABMV was also observed in their ability to induce local lesions on *C. amaranticolor* or *Ocimum basilicum*. Induction of local lesions by some strains of CABMV on *O. basilicum* had been reported (Lovisolo and Conti, 1966), but Fischer and Lockhart (1976) failed to get local lesions on *O. basilicum* by a Moroccan isolate of CABMV. In our repeated attempts to inoculate this species with CABMV-Mor and three other CABMV isolates we also failed to obtain local lesions. These results indicate that local lesions hosts could assist in the differentiation of CABMV variants. The bean and pea genotypes failed to differentiate CABMV variants.

The results of screening tests of 51 cowpea genotypes against 50 CABMV isolates indicated that sources of resistance to CABMV occur among currently promising U.S. cultivars and among TVU lines from IITA, Abadan, Nigeria. Immunity was more common than tolerance. This contrasts with the findings of Kaiser and Mossahebbi (1975), who failed to locate any source of immunity among 1054 lines in Iran tested against CABMV, although many (10.8%) were

considered resistant. However, use of a specific systemic rather than a general local lesion host might have permitted detection of lower concentration of virus.

In this study we have identified several lines/cvs to be immune, resistant or tolerant to individual isolates. Two TVU lines, TVU 401 and TVU 1582 were immune to all of the 50 CABMV isolates. Purple Hull Pinkeye-BVR was immune to all of the isolates except CABMV-Mor to which it was susceptible. Similarly, Serido and White Acre-BVR were also immune to all of the isolates except CABMV-Mor, and PI-39C. Ladipo and Allen (1979) tested 165 cowpea TVU lines against a Nigerian isolate of CABMV, and reported 52 immune and six tolerant to this isolate.

In this study we included some of the same TVU lines that had been previously investigated: (a) Ladipo and Allen (1979), tested against a Nigerian isolate of CABMV (b) Patel et al. (1982), tested against a Tanzanian isolate of CABMV (c) Taiwo et al. (1982), tested against four isolates of CABMV (Kenya, Nigeria, Morocco and Cyprus) and (d) Dijkstra et al. (1987) tested against four isolates of BlCMV and Morocco isolate of CABMV. Comparison of the reactions of selected cowpea genotypes to CABMV isolates compared to our results are shown in Table 4.7. In our study all of the 50 geographically diverse, seed-borne CABMV isolates failed to infect two TVU lines, TVU 401 and TVU 1582. The Nigerian isolate of Ladipo and Allen (1979)

Table 4.7: Comparison of the results of TVU differentials with CABMV isolates studied by various workers.

TVU genotypes	Workers and the CABMV isolates used in their study						
	Bashir Mor.	Ladipo[1] Nig.	Patel[2] Tanz.	Dijkstra[3] Mor.	Taiwo[4] Nig.	Kenya.	Mor.
TVU 109P2	+++	CLL/ss	+++	0	0	0	0
TVU 196	+++	-	+++	8/24(ss)	B-Mt	Mt	1/6[e]
TVU 347	+++	CLL/ss	+++	0	B-Mt	Mt	2/6
TVU 354	+++	0	+++	0	0	0	0
TVU 401	-	+/mixed	0	6/28(ss)	0	0	0
TVU 408P2	-	NLL/ss	+++	0	0	0	0
TVU 410	-	-/mixed	-	0	0	0	0
TVU 1000	+++	-/mixed	+++	0	0	0	0
TVU 1016-1	+++	-	+++	0	0	0	0
TVU 1582	-	-	+++	-	Mt.GVB	Mt.GVB	0
TVU 1593	-	-	0	-	Mt	Mt	0/5
TVU 2480	0	ss	+++	0	-	-	3/7
TVU 2657	+++	ss	+++	ss	-	-	VC[*]
TVU 2740	+++	ss	+++	Li	-	-	Mt
TVU 2845	+++	ss	+++	ss	-	-	Mt
TVU 3433	+++	ss	+++	mos	-	-	Mt

[1]Ladipo and Allen (1979). Tropical Agric. (Trinidad). 56: 353.
[2]Patel et al., 1982. Indian J.Genetic. 42: 221.
[3]Dijkstra et al., 1987. Neth. J. Pl. Path. 93: 115.
[4]Taiwo et al., 1982. Phytpath. 72: 590.

Symbols used to describe the symptoms induced by isolates:
- = Immune; +++ = Susceptible; 0 = Not tested; NLL = Necrotic local lesion; ss = Susceptible; Mixed = Some plants were infected and some not; Mos = Mosaic; B-Mt = Bright yellow mottle; GVB = Green vein banding; Li = Latent infection. 8/24 = Eight plants infected out of 24; 6/28 = Six plants infected out of 28.

also failed to infect these two lines. Two of the four isolates, Nigerian and Kenyan used by Taiwo et al (1982) infected these two lines; however these two isolates later proved instead to be BlCMV. Two other isolates, from Morocco and Cyprus, failed to infect these lines and were recognized as a CABMV isolates. The Tanzanian CABMV isolate of Patel et al. (1982) infected these two lines including two other TVU lines TVU 2657 and TVU 3433 which are resistant to all the BlCMV isolates except PU-7B. Later this CABMV isolate was recognized as a BlCMV isolate based on differential reactions of four TVU lines (P.N. Patel: personal communication and our results). The Morocco isolate of CABMV used by Dijkstra et al.(1987), failed to infect TVU 1582, while in the case of TVU 401, six out of 28 plants were infected. Among the TVU lines we identified four lines TVU 401, TVU 1582 (resistant to all CABMV isolates), TVU 2657 and TVU 3433 (resistant to all BlCMV isolates except PU-7B), established identities of 31/32 isolates of BlCMV and 50/50 isolates of CABMV. The Tanzanian isolate of CABMV (actually BlCMV) used by Patel et al (1982) is similar to BlCMV isolate PU-7B, found in the present study in a seed lot of Pusa Phalguni originating in India. The infected seeds of this variety were kindly provided by Dr.P.N. Patel, University of California, Riverside. The responses of these four TVU lines to inoculation with the CABMV-Mor isolate by Taiwo

et al. (1982), Dijkstra et al (1987) and by us were similar. Distinction between isolates of BlCMV from CABMV by these TVU lines was also reported by Taiwo et al.(1982).

Immunity, resistance and tolerance to a limited number of CABMV isolates had been reported (Kaiser et al., 1968, Kaiser and Mossahebbi, 1975 , Ladipo and Allen, 1975, Mali et al 1981, Mali et al., 1988). However, we report new sources of immunity, resistance, and tolerance to a large number of CABMV isolates. These genotypes should be of significant usefulness in resistance breeding programs. Two TVU lines, TVU 401 and TVU 1582 were not only immune to all the isolates of CABMV, but also distinguished BlCMV from CABMV isolates.

Differential reactions of 35 cowpea genotypes against 23 isolates (Table 4.4) showed that CABMV is variable in its pathogenicity and virulence. A wide range of patho-ogenic variation was found among the isolates of CABMV, which clearly indicated the existence of multiple variants of this virus. Some isolates (PI-44C, PK-16C, RN-14C) infected two to seven genotypes whereas other isolates (Mor, PI-39C, PI-40C, RN-18C) attacked 21-31 out of 35 genotypes. Likewise, some isolates induced very mild symptoms while the others were highly virulent. Some isolates induced localized infection only, while the others induced initial discrete lesions and subsequently induced systemic infection or necrosis. Taiwo (1982) who used only

two CABMV isolates (Kenyan and American), revealed differential responses of some of the TVU lines against these two isolates, indicating pathogenic variation among CABMV isolates. Bock (1973) also referred to CABMV strain variation. We report three groups of CABMV variants with distinct pathogenicity. Group 1, 2 and 3 included CABMV variants with very narrow to very wide range of pathogenicity. The variation among isolates may account for variable results of CABMV host range studies by various workers, and also illustrate the need for breeding programs developing CABMV-resistant cowpea cultivars to evaluate progeny against a range of CABMV isolates. Two lines, TVU 401 and TVU 1582, immune to all CABMV isolates are available to be included in breeding resistant cultivars.

CHAPTER-5

BIOLOGICAL CHARACTERIZATION OF SEED-BORNE ISOLATES OF BlCMV AND IDENTIFICATION OF BlCMV-IMMUNE GENOTYPES

ABSTRACT

Based on interactions of 51 cowpea genotypes and 21 BlCMV isolates, BlCMV variants were found to comprise a continuous pathogenicity gradient. The classification of pathogenic variants was not enhanced by inclusion of bean or pea genotypes or of local-lesion-producing host plant species.

Cowpea genotypes (e.g. White Acre-BVR, Serido and IT80S 2049) resistant to all BlCMV isolates were identified. Two TVU lines, TVU 2657 and TVU 3433, were immune to all BlCMV isolates except PU-7B, PU-8B and PU-10B isolates were derived from the same seed lot originating from India. Pusa isolates differed in their pathogenicity on TVU 2657 and TVU 3433 from all other isolates of BlCMV, and infected these two TVU lines which were resistant to all other isolates of BlCMV. Four TVU lines TVU 401, TVU 1582, TVU 2657 and TVU 3433 distinguished BlCMV from CABMV isolates.

These results clearly indicate that BlCMV and CABMV are two distinct potyviral entities.

INTRODUCTION

Forty-three isolates of BlCMV were derived from naturally infected seeds of U.S. *Vigna* pre-introductions and cowpea plants collected from commercially grown plots in southern California, U.S.A. A typical U. S. isolate of BlCMV, BlCMV-Ga, was obtained from Dr. C. W. Kuhn, Department of Plant Pathology, University of Georgia, Athens Ga, U.S.A. as a tentative type isolate for this study. Twenty-one of the 43 isolates were selected for a comparative study of their biological properties on 51 cowpea, 11 bean, and 7 pea genotypes, and three local lesion hosts with the following objectives: (a) to establish a set of cowpea differentials which could distinguish among BlCMV variants or BlCMV from CABMV. and (b) to identify resistant sources against BlCMV isolates to be included in breeding programs to develop resistant cultivars

MATERIALS AND METHODS

Virus isolates:

Twenty-one seed-borne BlCMV isolates were included in this study. These included three field isolates collected from commercial cowpea plots grown in Riverside area of California, and the Georgia type isolate BlCMV-Ga. All isolates were maintained by successive mechanical

inoculation on susceptible cowpea genotypes PI 218123, PI 251222 or Pusa Phalguni. Details concerning BlCMV isolates are presented in Appendix-2.

Source of cowpea genotypes:

The same cowpea genotypes tested against CABMV isolates were also tested against BlCMV isolates. The seed source of cowpea, bean, pea and local lesion hosts was the same as is described in Chapter-4.

Screening of cowpea genotypes for resistance:

Twenty-one BlCMV isolates were tested against 51 cowpea genotypes. The procedure for testing isolates against genotypes was the same as followed for CABMV, as described in Chapter-4. Local and systemic responses of cowpea genotypes to inoculation of primary leaves with each isolate were recorded weekly for five weeks, when the experiment was terminated. Symptomless plants were assayed by DAS-ELISA to differentiate immunity from tolerance.

Tests with bean and pea genotypes:

Seven isolates of BlCMV including BlCMV-Ga were tested on 11 genotypes of bean and 7 of pea. Local and systemic responses of each genotype to inoculation with each isolate was recorded at weekly intervals for 4 weeks post-inoculation. The screening procedure and assays were the same as described for CABMV isolates in Chapter-4.

Tests with local lesion hosts:

Three species (*C. amaranticolor, C. album and O. basilicum*) were evaluated as local lesion hosts for BlCMV isolates. Four leaves of each host were inoculated with each isolate and numbers of induced local lesions were recorded weekly for 3 weeks post- inoculation. Genotypes producing no local lesions were also tested by DAS-ELISA to detect any asymptomatic infection.

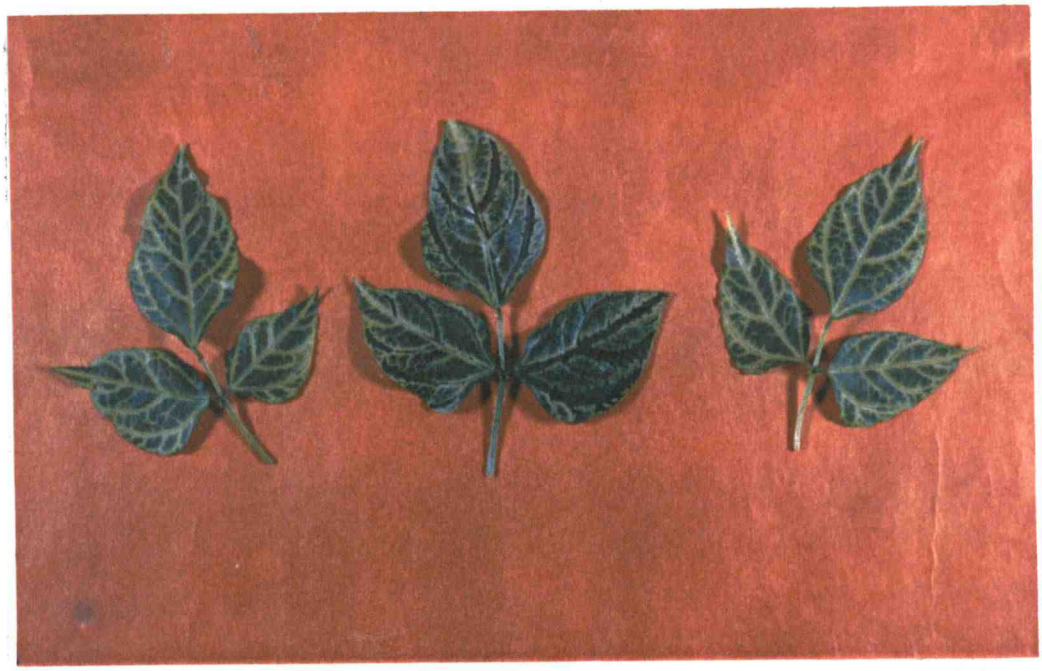
RESULTS

Symptomology and genotypic response:

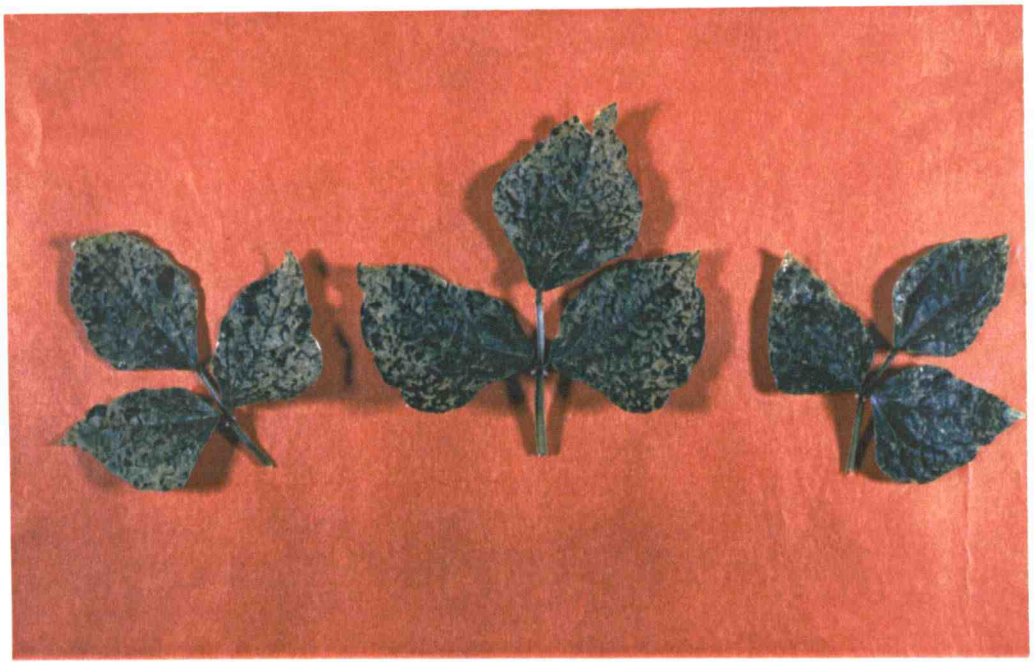
A wide variation in symptoms induced by each isolate was observed depending upon the virus isolate-genotype combinations (Table 5.1). Local symptoms induced by BlCMV isolates included large reddish and often ring-like lesions spread along the veins, forming a reddish-net pattern (Figure 6). Size of the local lesions varied from 0.2 to 3 mm in diameter. BlCMV-induced systemic symptoms consisted of severe mottling or mosaic, distortion and reddish vein necrosis. Systemic symptoms on other cowpea cultivars included mottle in which green bands along the veins commonly alternate with chlorotic or whitish interveinal areas together with some stunting and distortion (Figures 7). BlCMV in mixed infection with CMV induced severe stunting. A few isolates induced necrotic

Figure 6. Symptoms in cowpea genotype UCR 7964 induced by BlCMV isolate PU-7B. Note necrotic lesions and vein necrosis on inoculated primary leaves, followed by systemic mosaic symptoms.

Figure 7. Symptoms induced by two different isolates of BlCMV.



(a). Systemic symptoms induced by BlCMV isolate PU-7B in cowpea genotype 'Pusa Phalguni'. Note characteristic vein banding and vein chlorosis.



(b). Systemic mosaic in cowpea genotype PI 251222 induced by BlCMV-Ga isolate.

Table 5.1: Disease reactions of cowpea genotypes against BlCMV isolates 4 weeks after mechanical inoculation.

Genotype	Virus isolates													
	Ga		PI-3B		RF-4B		PU-6B		PU-7B		PU-8B		PU-10B	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	1		2		3		4		5		6		7	
TVU 109P2	.[a]	+++	.	+++	.	+++	.	++	.	++	.	+++	.	+++
TVU 196	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 347	.	+++	.	+++	.	+++	.	++	.	++	.	+++	.	+++
TVU 354	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 401	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 408P2	.	Li	.	-	.	-	.	-	.	-	.	-	.	-
TVU 410	.	Li	.	++	.	-	.	-	.	-	.	-	.	L
TVU 984	.	-	.	+++	.	-	.	+++	.	+++	.	++	.	L
TVU 1000	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	++
TVU 1016-1	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	++
TVU 1582	.	+++	.	+++	.	++	.	+++	.	+++	.	+++	.	+++
TVU 1593	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 2657	.	-	.	-	.	-	.	-	.	+++	.	+++	.	-
TVU 2740	.	-	.	-	.	-	.	-	.	+++	.	+++	.	+++
TVU 2845	.	+++	.	+++	.	+++	.	+++	o	o	.	+++	o	o
TVU 3433	.	-	.	-	.	-	.	-	.	+++	.	+++	.	+++
IT 80S 2049	.	-	.	-	.	-	.	-	.	-	.	-	.	-
CBE 3	.	-	.	Li	.	-	.	-	.	+++	NL,V	Ls	.	Li
CBE 5	.	+++	.	+++	.	Li	.	+++	NL	+++	NL,V	Ls	.	++
CBE 46	.	-	.	Li	.	-	.	-	NL	+++	NL,V	Ls	.	Li
CBE 88	.	-	.	-	.	-	.	-	NL	+++	NL,V	Ls	.	++

Cont,d..

Table 5.1: (Cont,d..)

Genotype	Virus isolates													
	Ga		PI-3B		RF-4B		PU-6B		PU-7B		PU-8B		PU-10B	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	1		2		3		4		5		6		7	
UCR 7964	.	-	.	Li	.	-	.	+++	NL	+++	NL,V	Ls	.	++
UCR 8517	.	-	.	Li	.	-	.	-	NL	+++	NL,V	Ls	.	-
UCR 8679	.	+++	.	Li	.	-	.	-	.	+++	.	-	.	-
UCR 160	.	++	o	o	.	Li	.	+++	o	o	NL	Ls	.	-
UCR 1393	.	++	o	o	.	Li	.	+++	o	o	NL	Ls	.	-
UCR 524B	.	++	o	o	.	Li	NL	+++	o	o	NL	Ls	.	-
P.P.H.BVR	.	-	.	-	.	-	.	-	o	o	.	-	.	-
White.A.BVR	.	-	.	-	o	o	o	o	o	o	.	o	o	-
Serido	.	-	.	-	.	-	.	-	.	-	.	-	.	-
Bettergreen	.	+++	.	+++	.	+++	.	+++	.	+++	o	o	o	o
M. Purple	.	-	.	-	.	-	.	-	o	o	o	o	.	-
M. Silver	.	-	.	-	.	-	.	++	.	++	.	Li	.	Li
Mopod	.	-	.	+++	.	+++	.	-	.	+++	NL	+++	.	++
Corona	.	-	.	-	.	-	.	-	.	-	.	-	.	-
Worthmore	.	-	.	-	.	-	.	-	.	+++	.	+++	.	Li
T.W.Crowder	.	+++	.	+++	.	-	.	-	.	+++	NL	Ls	.	++
T.Cream # 8	.	-	.	-	.	-	.	-	.	-	.	-	.	-
T.Cream # 40	.	+++	.	-	o	o	.	-	NL	+++	NL	Ls	.	++
Blue Goose	.	-	.	-	o	o	.	-	.	-	o	o	.	Li
M.Shipper	.	-	.	Li	.	-	.	-	.	+++	o	o	.	Li
Snappea	o	o	.	Li	o	o	o	o	.	-	o	o	.	-

Cont,d..

Table 5.1: (Cont,d..)

Genotype	Virus isolates													
	Ga		PI-3B		RF-4B		PU-6B		PU-7B		PU-8B		PU-10B	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	1		2		3		4		5		6		7	
Big Boy	.	-	.	-	.	-	.	-	.	-	.	-	.	-
B.S.Crowder	.	-	.	-	.	-	.	-	.	+++	.	Li	o	Li
M. Cream	.	-	.	o	.	-	.	-	.	o	o	o	.	-
Magnolia	.	-	.	Li	.	-	.	-	NL	+++	NL	+++	.	Li
K.P. Hull	.	+++	.	-	.	Li	.	++	.	+++	.	+++	.	Li
T. Pinkeye	o	o	o	o	o	Li	.	++	.	+++	.	-	o	o
Pusa Phalguni	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	++
PI 218123	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
PI 251222	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++

[a]**Symbols used for virus-induced symptoms in Table 5.1.** Cont,d..

. = No hypersensitive response (no local lesion production); o = Not tested; - = Immune, no visible symptoms and no virus detection by ELISA; + = Very mild systemic mosaic; ++ = Moderate type systemic mosaic, not much severe; +++ = Very clear characteristic virus symptoms (highly susceptible); Li = Latent infection, no visible symptoms, virus detected only by ELISA; NL = Necrotic local lesion (hypersensitive response); V = Vein necrosis on the inoculated primary leaves. Ls = Lethal susceptible, the plants were killled completely within two week after inoculation; Lo = Local reaction; Sy = Systemic reaction

Table 5.1: (Cont,d..)

Genotype	Virus isolates													
	PI-21B		PI-22B		PI-23B		PI-24B		PI-25B1		PI-25B2		PI-25B4	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	8		9		10		11		12		13		14	
TVU 109P2	.	+++	.	+++	.	+++	.	+++	.	++	.	+++	.	+++
TVU 196	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 347	.	++	.	+++	.	+++	.	+++	.	++	.	+++	.	+++
TVU 354	.	++	.	+++	.	+++	.	-	.	++	.	+++	.	++
TVU 401	.	++	.	+++	.	+++	.	+++	.	+++	.	+++	.	++
TVU 408P2	.	Li	.	Li	.	-	.	-	.	Li	NL,V	Li	.	Li
TVU 410	.	Li	.	-	.	-	.	-	.	Li	NL,V	Li	.	Li
TVU 984	.	+++	.	+++	.	+++	.	-	.	+++	.	+++	.	+++
TVU 1000	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 1016-1	.	+++	.	++	.	+++	.	+++	.	+++	.	Li	.	+++
TVU 1582	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	++
TVU 1593	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	o	o
TVU 2657	.	-	.	-	.	-	.	-	.	-	.	-	o	o
TVU 2740	.	-	.	-	.	-	.	-	.	-	.	-	o	o
TVU 2845	o	o	o	o	o	o	o	o	.	+++	.	+++	o	o
TVU 3433	.	-	.	-	.	-	.	-	.	-	.	-	o	o
IT 80S 2049	.	-	.	-	.	-	.	-	.	-	.	-	.	-
CBE 3	.	Li	.	-	.	Li	.	-	.	Li	o	o	.	Li
CBE 5	.	+++	.	++	.	Li	.	Li	.	Li	o	o	o	o
CBE 46	.	Li	.	-	o	o	.	-	.	Li	o	o	o	o
CBE 88	.	+++	.	-	.	Li	.	-	.	-	o	o	o	Li

Cont,d..

Table 5.1: (Cont,d..)

Genotype	Virus isolates													
	PI-21B		PI-22B		PI-23B		PI-24B		PI-25B1		PI-25B2		PI-25B4	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	8		9		10		11		12		13		14	
UCR 7964	NL	+++	.	-	.	+++	.	-	NL,V	+++	o	o	NL,V	-
UCR 8517	.	-	.	-	.	-	.	-	NL,V	+++	o	o	.	-
UCR 8679	.	-	.	Li	.	-	.	+++	.	-	o	o	.	-
UCR 160	.	++	.	++	.	+++	Li	++	NL	+++	o	o	o	Li
UCR 1393	.	++	.	++	.	+++	.	+++	.	Li	o	o	o	Li
UCR 524B	.	++	.	+++	.	++	NL	++	.	Li	o	o	o	Li
P.P.H.BVR	.	Li	.	-	o	o	.	Li	o	o	o	o	o	o
White.A.BVR		o	o	.	-	.	o	o	o	o	o	o	o	o
Serido	.	-	.	-	.	-	.	-	.	-	o	o	.	-
Bettergreen	.	+++	.	++	.	+++	.	+++	.	+++	o	o	o	o
M. Purple	.	-	.	Li	.	+++	.	-	o	o	o	o	o	o
M. Silver	.	-	.	Li	.	+++	.	-	o	o	o	o	o	o
Mopod	.	+++	.	+++	.	+++	.	+++	o	o	o	o	o	o
Corona	.	-	.	-	.	+++	.	-	o	o	o	o	o	o
Worthmore	.	+++	.	Li	.	-	.	-	o	o	o	o	o	o
T.W.Crowder	.	Li	.	-	.	Li	.	++	o	o	o	o	o	o
T.Cream # 8	.	-	.	-	.	-	Nl,V	Li	o	o	o	o	o	o
T.Cream # 40	.	-	.	-	NL,V	-	o	o	o	o	o	o	o	o
Blue Goose	.	-	.	Li	.	Li	o	o	o	o	o	o	o	o
M.Shipper	.	Li	.	Li	.	-	.	-	o	o	o	o	o	o
Snappea	.	-	.	-	.	-	o	o	o	o	o	o	o	o

Cont,d..

Table 5.1: (Cont,d..)

Genotype	Virus isolates													
	PI-21B		PI-22B		PI-23B		PI-24B		PI-25B1		PI-25B2		PI-25B4	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	8		9		10		11		12		13		14	
Big Boy	.	-	.	-	.	-	.	-	o	o	o	o	o	o
B.S.Crowder	.	-	.	-	.	-	.	-	o	o	o	o	o	o
M. Cream	.	-	.	Li	o	o	.	-	o	o	o	o	o	o
Magnolia	.	+++	.	++	.	+++	.	++	o	o	o	o	o	o
K.P. Hull	.	-	.	+++	.	Li	.	-	o	o	o	o	o	o
T. Pinkeye	o	o	.	+++	.	-	.	++	o	o	o	o	o	o
Pusa Phalguni	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	+++	+++
PI 218123	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	+++	+++
PI 251222	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	+++	+++

Cont,d..

Table 5.1: (Cont,d..)

Genotype	Virus isolates													
	PI-25B5		PI-25B6		PI-25B8		PI-25B9		RF-26B		RF-27B		COR-30B	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	15		16		17		18		19		20		21	
TVU 109P2	.	+++	.	++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 196	.	+++	.	++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 347	.	+++	.	++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 354	.	+++	.	+++	.	+++	.	+++	.	-	.	-	.	+++
TVU 401	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 408P2	.	Li	.	Li	.	Li	.	-	.	Li	.	Li	.	-
TVU 410	.	Li	.	Li	.	Li	.	-	.	++	.	++	.	-
TVU 984	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	-
TVU 1000	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 1016-1	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 1582	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 1593	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
TVU 2657	.	-	.	-	NL	-	.	-	.	-	.	-	.	-
TVU 2740	.	-	.	-	.	-	.	-	.	-	.	-	.	-
TVU 2845	.	+++	.	+++	.	+++	o	o	o	o	o	o	.	-
TVU 3433	.	-	.	-	.	-	.	-	.	-	.	-	.	-
IT 80S 2049	.	-	.	-	.	-	.	-	.	-	.	-	o	o
CBE 3	o	o	.	Li	o	o	.	Li	.	-	.	-	o	o
CBE 5	o	o	.	Li	o	o	.	Li	.	+++	NL,V	+++	o	o
CBE 46	o	o	.	Li	o	o	.	Li	.	-	.	-	o	o
CBE 88	o	o	.	Li	o	o	.	Li	.	-	.	-	.	-

Cont,d..

Table 5.1: (Cont,d..)

Genotype	Virus isolates													
	PI-25B5		PI-25B6		PI-25B8		PI-25B9		RF-26B		RF-27B		COR-30B	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	15		16		17		18		19		20		21	
UCR 7964	o	o	o	o	o	o	NL,V	-	NL	-	.	-	o	-
UCR 8517	o	o	.	-	o	o	.	-	NL		.	-	o	-
UCR 8679	o	o	.	-	o	o	.	-	.	-	.	-	o	++
UCR 160	.	++	.	Li	o	o	.	Li	NL	++	.	++	o	-
UCR 1393	.	++	.	Li	o	o	.	Li	.	++	.	++	o	++
UCR 524B	.	-	.	Li	o	o	NL	Li	.	+++	.	+++	o	++
P.P.H.BVR	.	-	o	o	o	o	.	-	.	-	.	-	.	-
White.A.BVR	.	-	o	o	o	o	.	-	.	-	.	-	.	-
Serido	.	-	.	-	o	o	.	-	.	-	.	-	.	-
Bettergreen	.	+++	o	o	o	o	.	+++	.	-	.	-	.	-
M. Purple	.	-	o	o	o	o	.	++	.	-	.	-	.	-
M. Silver	.	Li	o	o	o	o	o	o	o	o	.	-	.	-
Mopod	.	+++	o	o	o	o	.	+++	.	+++	.	+++	.	-
Corona	o	o	o	o	o	o	.	Li	.	+++	.	-	.	-
Worthmore	.	+++	o	o	o	o	.	Li	.	-	.	-	.	-
T.W.Crowder	.	-	o	o	o	o	.	-	.	-	.	-	.	-
T.Cream # 8	NL,V	Li	o	o	o	o	.	-	.	-	.	-	o	o
T.Cream # 40	.	o	o	o	o	o	.	++	.	-	.	-	o	o
Blue Goose	.	o	o	o	o	o	.	-	.	-	o	o	o	o
M.Shipper	.	Li	o	o	o	o	.	-	.	-	.	-	o	o
Snappea	.	o	o	o	o	o	o	o	.	-	.	-	o	o

Cont,d..

Table 5.1: (Cont,d..)

Genotype	Virus isolates													
	PI-25B5		PI-25B6		PI-25B8		PI-25B9		RF-26B		RF-27B		COR-30B	
	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.	Lo.	Sy.
	15		16		17		18		19		20		21	
Big Boy	.	-	o	o	o	o	.	-	.	-	.	-	.	++
B.S.Crowder	.	-	o	o	o	o	.	-	.	-	.	-	.	-
M. Cream	.	-	o	o	o	o	o	o	.	-	.	-	.	-
Magnolia	NL	+++	o	o	o	o	.	-	.	-	NL	-	.	-
K.P. Hull	.	-	o	o	o	o	.	-	.	++	.	-	o	+++
T. Pinkeye	.	++	o	o	o	o	.	Li	.	++	.	-	o	Li
Pusa Phalguni	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	o	+++
PI 218123	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++
PI 251222	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++	.	+++

lesions on inoculated plants followed by mild to severe mosaic. BlCMV isolate PU-8B was the only isolate among 21 which induced whole plant necrosis (lethal susceptibility) on 11 out of 51 genotypes.

Differential reactions and pathogenic variation:

Twenty-eight out of 51 cowpea genotypes produced reactions of value in differentiating 21 BlCMV isolates (Table 5.2). Differential response of genotypes to individual isolates was manifested as immune/resistant, tolerant or susceptible reactions. Cowpea differentials were arrayed into three groups on the basis of number of isolates attacking each group. Group 1, 2 and 3 were attacked by 1-3, 5-8, and 9-13 isolates respectively. Accordingly isolates were arrayed into four groups on the basis of their range of pathogenicity (number of genotypes attacked by each isolate). Group 1, 2, 3, and 4 included isolates attacking 4-5, 7-9, 13-16 and 19-21 genotypes respectively. A wide variation in the range of pathogenicity among the isolates was observed.

Isolate pathogenicity to bean and pea genotypes:

Of 11 bean and seven pea genotypes tested (Table 5.3) only Black Turtle Soup type-2 bean was susceptible to systemic infection by any BlCMV isolate. Six of seven BlCMV isolates induced mild to severe systemic mosaic in this genotype following initial necrotic lesions with vein necrosis on primary inoculated leaves. The other 10

Table 5.2: Differential reactions of BlCMV isolates induced in cowpea genotypes and arranged according to their pathogenicity.

Cultivar	Host group	BlCMV variants groups																No of variants attacking each CV.
		I		II					III						IV			
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
Big Boy	1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
TVu 2740		-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	2
Corona		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	2
T.Cream #8		-	-	-	-	t	-	-	-	-	-	-	-	t	-	-	-	2
TVU 2657		-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	3
TVU 3433		-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	3
UCR 8517		-	-	-	-	-	-	-	t	-	-	-	-	-	-	+	+	3
M.Purple		-	-	-	-	-	-	-	-	t	+	+	-	-	-	-	-	3
B.S.Crowser		-	-	-	-	-	-	-	-	-	-	-	-	-	t	t	+	3
UCR 8679		-	+	-	-	-	-	+	t	t	-	-	-	-	-	-	+	5
CBE 48	2	-	-	-	-	-	-	-	-	-	t	t	+	-	+	+	+	7
TVU 408P2		t	-	-	-	-	t	t	-	t	-	t	t	+	-	-	-	7
Worthmore		-	-	-	-	-	-	-	-	t	-	t	t	+	t	+	+	7
CBE 3		-	-	-	-	-	-	-	t	-	t	t	+	t	t	+	+	8
TVU 410		+	-	-	-	-	+	t	+	-	-	t	t	t	t	-	-	8
M. Silver		-	-	-	+	-	-	-	+	t	+	t	t	t	t	+	t	8
UCR 7964		-	-	-	+	-	-	-	t	-	+	+	+	-	+	+	+	8
T.W.Crowder		-	-	-	-	+	-	+	+	-	t	-	t	-	+	+	+	8
M. Shipper		-	-	-	-	-	-	-	t	+	t	-	t	t	t	+	+	8
CBE 46		-	+	t	-	-	-	-	t	-	-	t	t	t	t	+	+	9

Cont,d..

Table 5.2: (Cont.d..)

Cultivar	Host group	BlCMV variants groups																No of variants attacking each CV.
		I				II						III					IV	
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
Magnolia	3	+	-	-	-	+	-	-	t	+	+	-	+	+	t	+	+	10
K.P.Hull		-	+	t	+	-	+	+	-	+	+	-	-	-	t	+	+	10
T.Pinkeye		-	t	t	+	+	-	+	-	+	t	t	+	+	-	-	+	10
TVU 984		+	-	-	+	-	+	-	+	+	+	+	+	+	t	+	+	12
UCR 160		+	-	-	+	+	+	+	-	+	+	t	+	+	-	+	+	12
Mopod		+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	12
B.green		-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	13
TVU 354		-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	13
		4	5	7	8	8	8	9	13	14	15	15	16	16	19	20	21	

- : Immune t : Tolerent + : Susceptible

A. RF-27B E. PI-24B I. PI-22B M. PI-25B5
B. Cor-30B F. RF-26B J. PI-23B N. PI-10B
C. RF-4BB G. CABMV-Ga K. PI-25B9 O. PU-8B
D. PU-6B H. PI-3B L. PI-21B P. PU-7B

Table 5.3: Response of bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*) genotypes against BlCMV isolates.

Host genotype	Virus isolates							
	Ga	3B	7B	8B	22B	26B	25B	BCMV(TS)
A. Beans								
Red Kidney	NL,V	NL,V	-	-	-	-	NL,V	+++
Hystyle	-	-	-	-	-	-	-	-
Dwarf Hort.	-	-	-	-	-	-	-	+++
Great Nort.	NL,V	-	NL,V	NL,V	-	NL,V	NL,V	-
91 G	-	-	-	-	-	-	-	-
Pinto	NL,V	NL,V	NL,V	NL,V	-	-	NL,V	-
B.T.Soup-1	-	-	-	-	-	-	-	-
B.T.Soup-2	NL/+	++	NL/+	NL/+	++	NL,V	++	+++
Bountiful	-	-	-	-	-	-	-	+++
Stringless Green Refugee	-	-	-	-	o	-	-	+++
B. Peas								
Ceras	-	-	-	-	0	-	-	0
Charger	-	-	-	-	0	-	-	0
Dual	-	-	-	-	0	-	-	0
Sounder	-	-	-	-	0	-	-	0
D.G. Sugar	-	-	-	-	0	-	-	0
Badger	-	-	-	-	0	-	-	0
15491	-	-	-	-	0	-	-	0

[a] **Symbols used for virus-induced symptoms in Table 1.**

- = Immune, no symptoms and no ELISA detectable virus; + = Mild systemic mosaic; +++ = Sever systemic mosaic (susceptible); NL = Necrotic lesions; V = Vein necrosis in inoculated leaves; L = Latent infection (ELISA detectable virus); st = Stunting; 0 = Not tested.

genotypes were systemically resistant (no ELISA-detectable virus) to all isolates, but virus was detected in inoculated leaves irrespective of symptoms. Three genotypes, Red-kidney, Great-Northern, and Pinto produced necrotic lesions with a reddish net of vein necrosis when inoculated with some BlCMV isolates. All pea genotypes were immune to the tested isolates of BlCMV.

Tests for Local lesions:

Three BlCMV isolates, PU-6B, PU-8B and PI-21B induced no local lesions on *C. amaranticolor*. The other isolates differed significantly in number of local lesions they induced (Table 5.4). The least number of local lesions (4) were induced by BlCMV-Ga isolate, while PI-22B induced 50. Three isolates tested on *C. album* induced 18-32 local lesions. Only one isolate BlCMV-Ga out of four produced local lesions on *O. basilicum* type-1. The other three isolates (PI-3B, PU-7B and PU-8B) induced no local lesions on any basil genotype.

Immunity to BlCMV- isolates:

Cowpea genotypes were either immune, resistant or tolerant to BlCMV isolates (Table 5.5). Two cowpea genotypes, IT 80S 2049 and White Acre-BVR were immune to all the BlCMV isolates tested, whereas TVU 2657, TVU 2740 and TVU 3433 were immune to all except Pusa isolates (PU-7B, PU-8B, PU-10B) of BlCMV. Resistant genotypes were also identified against individual BlCMV variants.

Table 5.4: Local lesions induced by BlCMV isolates.

Isolate	Host	No. of local lesion
BlCMV-Ga	*C. amaranticolor*	4[a]
PI-3B	"	32
PU-6B	"	0
PU-8B	"	0
PI-21B	"	0
PI-22B	"	50
PI-24B	"	20
PI-25B1	"	16
RF-26B	"	18
RF-27B	"	35
COR-30B	"	25
BlCMV-Ga	*C. album*	32
PI-3B	"	40
PI-22B	"	18
	Ocimum basilicum	
BlCMV-Ga	Basil (Tupe-1)	4
	" (Type-2)	0
	" (Type-3)	0
	" (Type-4)	0
PI-3B	Basil (Tupe-1)	0
	" (Type-2)	0
	" (Type-3)	0
	" (Type-4)	0
PU-7B	Basil (Tupe-1)	0
	" (Type-2)	0
	" (Type-3)	0
	" (Type-4)	0
PU-8B	Basil (Tupe-1)	0
	" (Type-2)	0
	" (Type-3)	0
	" (Type-4)	0

[a] Number represents average value of local lesions produced in four leaves of each species.

0 : No local lesion induced.

Table 5.5: Grouping of cowpea genotypes based on their reactions against BlCMV isolates.

Reaction type	Genotypes falling under each reaction type
Immune (4) (To all isolates)	TVU 2657[1], TVU 2740[1] , TVU 3433, White Acre-BVR, IT 80S 2049.
Resistant/Tolerant (30)	TVU 354, TVU 408P2, TVU 410, TVU 984, TVU 2657, CBE 3, CBE 46, CBE 88, UCR 7964, UCR 160, UCR 8517, UCR 8679, Mississippi Purple, Mississippi Silver, Purple Hull Pinkeye-BVR, Mopod, Corona, Worthmore, Texas White Crowder, Texas cream, Texas Cream # 8, Texas Cream # 40, Blue Goose, Brown Sugar Crowder, Mississippi Cream, Magnolia, Knuckle Purple Hull, Texas Pinkeye.
Susceptible (14) (to all isolates)	TVU 109P2, TVU 196, TVU 347, TVu 401, TVU 1000, TVU 1016-1, TVU 1582, TVU 1593, CBE 3, UCR 1593, UCR 524B, Pusa Phalguni, PI 218123, PI 251222.

[1]These genotypes are susceptible to BlCMV Pusa isolates (Pu-7B, PU-8B and PU-10B)

DISCUSSION

BlCMV could not be distinguished from CABMV on the basis of symptomology, because isolates of both viruses induced similar symptoms in the same cowpea genotypes. A Nigerian strain of CABMV had been reported to induce green veinbanding in infected cowpea (Ladipo, 1976), a symptom also commonly induced by BlCMV isolates as indicated in the present study of BlCMV in cowpea genotypes. Some isolates (PI-23B, PI-25B4, PI-25B9, RF-26B, RF-27B) induced hypersensitive response in four to five cowpea genotypes. Isolate PI-25B4 induced necrotic rings on inoculated leaves of UCR 7964, which is similar to that was reported for a strain of BlCMV from South Carolina (Murphy et al., 1987) and two potyvirus isolates from *Phaseolus vulgaris* (Makkouk at el., 1986).

Symptoms are affected dramatically by combinations of cowpea genotypes and virus strain (Kuhn, 1990). Data from this study verify the undependable nature of symptomology, as a criterion for virus identification, precise diagnostic methods. It is also undesirable to base virus "resistance" only on an absence of symptoms. We observed several inoculated genotypes that were completely free of symptoms but contained high concentrations of virus when tested by ELISA.

BlCMV isolates differed greatly in their range of

pathogenicity and virulence and induced differential reactions depending upon cowpea genotypes. Taiwo et al. (1982), using virus reactions to a number of IITA-TVU cowpea lines, were able to clearly differentiate between BlCMV and CABMV. We confirm their results regarding the usefulness of these TVU lines to distinguish BlCMV and CABMV. There was only one exception that all Pusa isolates (all of the isolates derived from the same seed lot of Pusa Phalguni) except PU-6B infected these TVU lines which were immune to all other isolates of BlCMV. This seed-borne isolate was unique in its pathogenicity and behaved like CABMV isolates on TVU 2657 and TVU 3433 which are susceptible to CABMV and resistant to other BlCMV isolates. Patel et al. (1982) tested a Tanzanian CABMV isolate on these four TVU lines (which differentiate BlCMV and CABMV) also infected these four TVU lines. BlCMV and CABMV could be distinguished by using TVU 401, TVU 1582, (resistant to all CABMV isolates), TVU 2657, TVU 2740, and TVU 3433 (resistant to all BlCMV isolates), but we could not depend totally on these lines for differentiation of both viruses unless confirmed by serology, because we found some isolates of CABMV were unable to infect TVU 2657 and TVU 3433 (which are susceptible to most of the CABMV isolates) and some isolates of BlCMV were able to infect TVU 2657, TVU 2740 and TVU 3433 (which are immune to most of the BlCMV isolates).

Resistance is widely recognized as the best control measure for plant viruses. Although resistant sources to a limited number of BlCMV isolates had been reported (Lima et al., 1979; Collins et al., 1985; Taiwo et al., 1982, Kuhn, 1990; Mali et al., 1988), we report some new resistant sources to several geographically diverse isolates of BlCMV. Out of 51 cowpea cultivars/lines tested against 21 BlCMV isolates, three genotypes, White Acre-BVR, Serido and IT 80S 2049 were immune to 10, 19 and 20 isolates respectively tested. A promising cultivar, Pinkeye Purple Hull-BVR was tested against 14 isolates and showed immunity to 12. This cultivar was tolerant to PI-21B and PI-24B. Several promising U.S. cultivars showed immunity, resistance, and tolerance to individual isolates (Table 5.1). Serido was immune to all 21 isolates of BlCMV, thus being a good candidate for a breeding program.

Among the TVU lines TVU 2657, TVU 2740 and TVU 3433 were immune to all except Pusa isolates (PU-7B, PU-8B, PU-10B), which were derived from an infected seed lot of Pusa Phalguni originated from India. We tested 25 Pusa isolates on TVU 2657 and TVU 3433; all of these isolates infected these two lines except PU-6B, which behaved like all other BlCMV isolates. Similarly TVU 2740 was also immune to all except Pusa isolates (PU-7B, PU-8B, PU-10B). The resistance of these TVU lines to BlCMV isolates had been reported (Taiwo et al., 1982, Dijkstra et al., 1987

and Huguenot et al., 1992). The high level of resistance in cowpea TVU lines represents valuable germplasm material that can easily be used in breeding programs for developing new viral resistant cultivars of *Vigna unguiculata*. In addition to TVU 2657, TVU 3433, and TVU 2740 some other TVU lines also showed resistance against individual isolates (Table 5.1).

In this study we could not find any cowpea genotype resistant to all isolates of BlCMV and CABMV, which indicate that no common sources of resistance are present to these two potyviruses, and our findings are the same as reported by Taiwo et al. (1982).

Bean and pea genotypes used in this study contributed little to characterizing BlCMV variants, which differs with the suggestion of Huguenot et al. (1992) that susceptibility of *Psaseolus vulgaris* to BlCMV could facilitate distinction from CABMV isolates (G.I. Mink: personal communication). However, *P. vulgaris* genotypes were not specified. None of the 11 *P. vulgaris* genotypes proved useful as differential hosts for viruses or viral variants.

We confirmed the results obtained by Taiwo et al. (1982) that the two viruses BlCMV and CABMV are distinct viral entities. BlCMV and CABMV were also clearly distinguished by DAS-ELISA (see Chapter-7). These results clearly indicate that BlCMV and CABMV are two distinct viruses, and our results are in agreement with those

reported by Lima et al. (1979); Taiwo et al. (1982) and Huguenot et al. (1992). Since it has been found that in addition to United States BlCMV also occurs in Nigeria, India, Japan, Afghanistan, Pakistan, and Tanzania (Taiwo et al., 1982; Bashir and Hampton, 1991; Mali et al., 1988; Huguenot et al., 1992), it is conceivable that BlCMV and CABMV already exist wherever cowpea crop is cultivated. Both viruses are seed-borne and are readily transmitted by several species of aphids. The free exchange of seed has probably facilitated their dissemination to regions in which one or both previously may have been absent. Thus control of both BlCMV and CABMV can best be implemented by incorporating separate resistance genes into improved cowpea cultivars.

CHAPTER-6

SEED AND APHID TRANSMISSION OF SELECTED ISOLATES OF BlCMV AND CABMV

ABSTRACT

Rates of seed-transmission was investigated for 10 isolates of BlCMV and 12 isolates of CABMV in three or four cowpea genotypes. Seed-transmission rates varied similarly between viruses, and significantly differed among isolates, and among cowpea genotypes. The highest seed transmission rate demonstrated for BlCMV, 48.5%, occurred in seeds from plants of 'Pusa Phalguni' that had been inoculated with isolate PI-25B1. 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 '58-57', the cowpea genotype from which the isolate was initially obtained. CABMV isolates RN-28C and PI-44C were also seed-transmitted at rates of ~29.7 and 33.8% respectively in this genotype. Approximately one-third of the isolate-genotype combinations, for both BlCMV and CABMV, resulted in no measurable seed-transmission of virus.

Results of non-persistent aphid-transmission were determined for three selected isolates each of BlCMV and CABMV, using three aphids (*Aphis craccivora* Koch) per

inoculated cowpea plant of a selected genotype. Average transmission rates of the two viruses were comparable, ranging from 24% to 55% among three BlCMV isolates, and from 18% to 57% among three CABMV isolates.

INTRODUCTION

Seed infection plays a major role in both the survival and perpetuation of viral pathogens. In ecological terms, seed transmission provides an ideal means by which infection foci are established in planted fields and from which secondary disease spread may occur. In addition to the importance of seed infection to local spread of virus, seed transmission is instrumental in long-distance dissemination during international exchanges of crop seeds or germplasm. Of the several viruses reported to be seed transmitted in cowpea (Hampton, 1983), BlCMV and CABMV are usually the most often encountered. Seed transmission of BlCMV dependent on cowpea cultivars and viral isolates, has been reported to range from 3.5% to 55.0% (Anderson, 1955; Zettler and Evans, 1980; Mali and Kulthe, 1980a; Mali et al., 1983; Mali et al., 1988). The level of CABMV seed transmission also varies among cultivars and virus isolates and ranged from 0.0% to 40% (Phatak, 1974; Bock and Conti, 1975; Ladipo, 1977; Aboul-Ata et al., 1982). Cowpea lines resistant to BlCMV or CABMV seed transmission

have been reported (Ladipo, 1977; Mali et al., 1983; Zettler and Evans, 1972).

Aphids play an important role in the secondary spread of potyviruses, after establishment in the field. Several aphid species such as *Aphis craccivora*, *A. gossypii*, *Macrosiphum solinifoli*, *Myzes persicae*, *A. fabae*, *A. medicaginis* and *M. euphorbiae* have been reported to non-persistently transmit either BlCMV or CABMV (Anderson, 1955; Mali and Kulthe, 1980a; Pio-Ribero et al., 1978; Zhao et al., 1991a; Vidano and Conti, 1965; Bock, 1973; Atiri et al.,1984). Transmission rates vary with minimum required acquisition access feeding period depending upon investigators, aphid species/biotype, and virus isolate (Matthews, 1992). The purpose of this study was to determine the variability in rates of seed and aphid transmission for selected BlCMV and CABMV isolates, and to verify the conventional non-persistent mode of transmission of BlCMV and CABMV isolates derived from infected seeds.

MATERIALS AND METHODS

Seed transmission:

The virus isolates used in this investigation were originally derived from infected seeds of cowpea pre-introductions and seed lots from Pakistan. All isolates were maintained by successive mechanical inoculation in

susceptible cowpea genotypes under insect-free greenhouse conditions. For the seed-transmission study twenty seeds of three selected cowpea genotypes (10 - 15 seeds per pot, 10 - 20 pots or more per genotype, depending upon the number of seeds available) were germinated under greenhouse conditions. When seedling primary leaves were fully expanded, the plants were mechanically inoculated respectively with selected isolates. Non-inoculated plants were included in each case as controls. Infected plants were allowed to grow to maturity and seed from infected and control plants were harvested.

Seeds harvested from infected and control plants (numbers varied among treatments) were planted in plastic pots filled with sterilized soil, and allowed to grow under insect-free greenhouse conditions. The growing seedlings were observed for virus-like symptoms. Symptomless plants were assayed by ELISA to detect possible latent infection. Final observations were recorded four weeks after planting. Selected symptomatic seedlings were tested by DAS-ELISA to verify BlCMV or CABMV symptomology. The percentage of seed transmission was based on symptomatic seedlings plus DAS-ELISA-positive asymptomatic seedlings.

Aphid transmission:

Three isolates each of BlCMV (Ga, PU-7B and PI-3B) and of CABMV (RN-7C, RN-27C and PI-39C) were tested to determine their transmissibility by *Aphis craccivora*. The

colony of *A. craccivora* was reared on healthy cowpea plants under controlled conditions. Plant-to-plant transmission was carried out as follows: Groups of 20 to 50 *Aphis craccivora* apterae, previously fasted for 2 hr, were deposited on detached, infected cowpea (PI 218123) leaves and closely observed for several minutes. Aphids that had fed normally for 3 to 4 min were carefully transferred to an expanding trifoliate leaf of PI 218123 seedlings, three aphids per plant. Twenty-seven to 45 plants were thus inoculated with each BlCMV or CABMV isolate. Aphids were then allowed to feed on test plants overnight before being killed by aerosol fumigation (synthetic pyrethroid; fumigation chamber). Inoculated plants were observed for symptom development for four weeks after exposure to aphids. Symptomless plants were assayed by ELISA.

RESULTS

Seed transmission:

Seed transmission rates varied among combinations of virus isolates and cowpea genotypes, ranging from 0.0% to 55%. The highest seed transmission rate, 48.5%, occurred in seeds of Pusa Phalguni plants that had been inoculated with BlCMV isolate PI-25B1. Genotypes were seed-transmitted at rates of 43% and 42% PI-22B and PI-3B respectively in Pusa Phalguni cowpea cultivar (Table 6.1). Four isolates of

Table 6.1: Seed transmission of selected BlCMV isolates in cowpeas genotypes.

Isolate	Genotype	Percent seed transmission.	
BlCMV-Ga	PI 218123	10[1]/308	(3.2)[2]
	PI 251222	0/110	(0.0)
	Pusa Phalguni	4/115	(3.5)
PI-3B	PI 218123	0/210	(0.0)
	PI 251222	4/105	(3.8)
	Pusa Phalguni	27/65	(41.5)
RF-4B	PI 218123	3/34	(8.8)
	PI 251222	9/272	(3.3)
	Pusa Phalguni	1/50	(2.0)
PU-7B	PI 218123	0/90	(0.0)
	PI 251222	0/70	(0.0)
	Pusa Phalguni	50/230	(21.7)
PI-21B	PI 218123	0/115	(0.0)
	PI 251222	0/92	(0.0)
	Pusa Phalguni	0/205	(0.0)
PI-22B	PI 218123	3/112	(2.7)
	PI 251222	0/90	(0.0)
	Pusa Phalguni	30/70	(42.8)

Cont,d..

Table 6.1 (Cont,d..)

Isolate	Genotype	Percent seed transmission.	
PI-23B	PI 218123	0/92	(0.0)
	PI 251222	0/201	(0.0)
	Pusa Phalguni	20/66	(30.3)
PI-24B	PI 218123	1/119	(0.8)
	PI 251222	0/107	(0.0)
	Pusa Phalguni	15/69	(21.7)
PI-25B1	PI 218123	0/87	(0.0)
	PI 251222	0/110	(0.0)
	Pusa Phalguni	16/33	(48.5)
RF-26B	PI 218123	4/210	(1.9)
	PI 251222	0/117	(0.0)
	Pusa Phalguni	10/36	(27.8)

[1]Number of seedlings developed symptoms or reacted positively in DAS-ELISA / total seedlings examined.

[2]Values in parenthesis are percent seed transmission.

BlCMV, PU-7B, PI-21B, PI-23B and PI-25B1 were not seed transmissible in genotypes PI 218123 and PI 251222, which are most susceptible to almost all BlCMV isolates. Other isolates were transmitted through seeds of these two genotypes at low rates (0.8 to 8.8%).

CABMV isolate RN-27C was seed-transmitted at a rate of 55% in its original genotype (58-57), followed by rates of 33.8% and 29.7% for PI-44C and RN-28C, respectively, in their original genotypes from which they were isolated (Table 6.2). These three isolates (RN-27C, RN-28C and PI-44C) were not transmitted through seeds of PI 218123 and PI 252222. Two isolates (RN-14C and PI-44C) were not seed transmitted in three susceptible cowpea genotypes (PI 218123, PI 251222 and Pusa Phalguni). The symptoms on primary and trifoliate leaves of infected seedlings were typical of those induced by BlCMV and CABMV, and identities of the viruses were verified by DAS-ELISA.

Aphid transmission:

All six isolates, three of each BlCMV and CABMV were easily transmitted non-persistently by *A. craccivora* to susceptible cowpea genotype PI 218123. However, significant variation in transmission rates occurred among isolates of BlCMV and CABMV (Table 6.3) The highest rate of transmission, 54.6%, occurred with PI-3B, followed by 46.3 and 24.1% with BlCMV-Ga and PU-7B respectively. The highest aphid-transmission rates among CABMV isolates,

Table 6.2: Seed transmission of selected CABMV isolates in cowpeas genotypes.

Isolate	Genotype	Percent seed transmission.	
RN-4C	PI 218123	5/104	(4.8)
	PI 251222	0/210	(0.0)
	Pusa Phalguni	12/202	(5.9)
RN-7C	PI 218123	6/108	(5.6)
	PI 251222	1/80	(1.3)
	Pusa Phalguni	41/200	(20.5)
RN-10C	PI 218123	2/103	(2.9)
	PI 251222	0/43	(0.0)
	Pusa Phalguni	41/215	(19.0)
RN-11C	PI 218123	0/81	(0.0)
	PI 251222	5/93	(5.3)
	Pusa Phalguni	10/65	(15.4)
RN-14C	PI 218123	0/87	(0.0)
	PI 251222	0/68	(0.0)
	Pusa Phalguni	0/59	(0.0)
PI-23C	PI 218123	5/123	(3.8)
	PI 251222	3/230	(1.3)
	Pusa Phalguni	24/175	(13.7)

Cont,d...

Table 6.2 (Cont,d..)

Isolate	Genotype	Percent seed transmission.	
RN-27C	PI 218123	0/110	(0.0)
	PI 251222	0/230	(0.0)
	58-57	34/65	(54.8)
RN-28C	PI 218123	0/98	(0.0)
	PI 251222	0/67	(0.0)
	U-449	11/37	(29.7)
PI-44C	PI 218123	0/113	(0.0)
	PI 251222	0/215	(0.0)
	Pusa Phalguni	0/42	(0.0)
	PI 302458	40/118	(33.8)
PK-1C	PI 218123	2/107	(1.8)
	Local	5/90	(5.5)
PK-4C	PI 218123	0/215	(0.0)
	Local	7/112	(6.3)
PK-12C	PI 218123	3/215	(1.4)
	Local	6/119	(5.0)

[1]Number of seedlings developed symptoms or reacted positively in DAS-ELISA / total seedlings examined.

[2]Values in parenthesis are percent seed transmission.

Table 6.3: Transmission of seed-borne isolates of BlCMV and CABMV by *Aphis craccivora* in cowpea genotype.

Virus	Isolate	Experiment			Average percent transmission
		1	2	3	
BlCMV	Ga	9[1]/40 (22.5)	18/32 (56.3)	21/35 (60.0)[2]	46.3 b[3]
	PU-7B	5/27 (18.5)	13/45 (28.8)	8/32 (25.0)	24.1 a
	PI-3B	21/41 (51.2)	21/35 (60.0)	19/36 (52.8)	54.6 b
CABMV	RN-7C	12/35 (34.3)	25/45 (55.5)	29/43 (67.5)	52.4 b
	RN-27C	4/23 (17.0)	5/36 (13.8)	8/35 (22.0)	17.6 a
	PI-39C	22/40 (55.0)	24/35 (68.5)	13/28 (46.4)	56.6 b

[1]Number infected per number tested; 3 aphids per plant.

[2]Values in parenthesis are percent transmission.

[3]Treatments followed by different letters (a,b) in the same column are significantly different (P = 0.05) according to Duncan's multiple range test.

56.6% was obtained with isolate PI-39C, followed by RN-7C (52.4%). Isolate RN-27C was transmitted at a significantly lower rate of 17.6%.

DISCUSSION

BlCMV and CABMV are important potyviruses since they are known to be seed transmitted in cowpea (Bock and Conti, 1974; Purcifull and Gonsalves, 1985). Seed transmission has been reported to range from 0.3% (Aboul-Ata et al., 1982) to 55% (Mali et al., 1983; Zettler and Evans, 1972). In our studies BlCMV isolate PI-25B1 was seed transmitted at rates 0.0% to 48.5% among three cowpea genotypes. Seed-transmission rates of CABMV isolate RN-27C likewise ranged from 0.0 to 54.89% among three genotypes. In both cases, high seed-transmission rates were confined to the genotypes from which the seed-borne isolates were taken. This and other evidence from these studies suggest that (a) some isolates exist primarily by virtue of their adaptive seed-transmissibility in specific genotypes, and (b) that those unique viral-isolate/ *Vigna*-genotype association may represent a long-standing relationships, with gradual viral specialization towards seed-transmissibility.

Aphid vectors are important for potyviruses in their secondary spread of viruses from initial foci (e.g., infected seed/seedlings). Several species of aphids have

been reported to transmit BlCMV and CABMV (Bock, 1973; Bock and Conti, 1874; Purcifull and Gonsalves, 1985). In our results all of six isolates of BlCMV and CABMV tested were readily transmitted by *A. craccivora*. However, isolates differed significantly in their transmissibility by *A. craccivora*. BlCMV isolate PU-7B (24.1%) and CABMV isolates RN-27C (17.6%) were transmitted at low rates as compared to others. Aphid transmission of potyviruses is influenced by many factors, and involves remarkable virus-isolate /aphid-biotype specificity. Dependence on a helper component for aphid transmission is now considered a general feature of potyviruses (Pirone, 1991; Hollings and Brunt, 1981). In the present study, we found that the CABMV isolate RN-27C, which was seed transmitted at high rate (55%) in its original genotype (58-57), was aphid transmitted at a low rate (17.6%). It is conceivable that a high degree of adaptive seed-transmissibility could lessen selection pressure for aphid-transmissibility in the perpetuation of such potyviral variants.

CHAPTER-7

SEROLOGY OF SEED-BORNE ISOLATES OF BlCMV AND CABMV, AND THEIR RELATIONSHIPS WITH OTHER POTYVIRUSES

ABSTRACT

Fifty-seven seed-borne geographically, diverse potyviral isolates derived from *Vigna* pre-introductions and seed lots were compared serologically, including type isolates of BlCMV (BlCMV-Ga) and CABMV (RN-7C) and CABMV-Mor and their respective homologous antisera. DAS-ELISA results clearly partitioned the isolates into two distinct groups: 20 that reacted exclusively with anti-BlCMV IgG and 37 that reacted exclusively with anti-CABMV IgG. This grouping of potyviral isolates was consistent based on serological and biological properties. The same distinction of BlCMV isolates from CABMV isolates was obtained by SDS-immunodiffusion tests, where patterns of precipitin-bands of BlCMV isolates spurred over CABMV isolates, when antiserum to BlCMV was used. All BlCMV isolates showed no reaction in DAC-ELISA and SDS-immunodiffusion tests, when tested with CABMV antiserum.

Three monoclonal antibodies, I-Z, II-197 and II-463 (Wang et al., 1984), produced to BCMV epitopes were also used to compare 20 isolates of BlCMV and 32 of CABMV. MAb I-Z did not react to any isolate of BlCMV or CABMV.

MAb II-197 recognized all isolates of BlCMV and CABMV as well as other potyviruses, whereas MAb II-463 reacted decisively with BlCMV isolates, but did not react with isolates of CABMV.

Based on DAS-ELISA, SDS-immunodiffusion, monoclonal antibody tests and biological behavior on selected cowpea genotypes it was concluded that BlCMV and CABMV are two distinct potyviral entities.

INTRODUCTION

BlCMV was first reported by Anderson (1955), and CABMV was described 10 years later by Lovisolo and Conti (1966). Both viruses are members of the potyvirus group (Matthews, 1979: Hollings and Brunt, 1981). Initially there was some confusion about the status of BlCMV. BlCMV was assumed to be a strain of bean yellow mosaic virus (BYMV) (Corbett, 1956; Kuhn et al., 1966), or a strain of CABMV (Martyn, 1968). CABMV was assumed to be a synonym of BlCMV (Edwardson, 1974). Uyemoto et al. (1973) reported that BlCMV and bean common mosaic virus (BCMV) were serologically related. As a result of serological and cytological studies (Lima et al., 1978, 1979), BlCMV was concluded to be none of the above, but a distinct member of the potyvirus group (Matthews, 1979; Hollings and Brunt, 1981). Although serological cross reactions can occur

among various members of the potyvirus group depending upon serological tests (Hollings and Brunt, 1981), BlCMV and CABMV were regarded as two distinct potyviruses (Lima et al., 1979; Matthews, 1979; Taiwo et al., 1982; Taiwo and Gonsalves, 1982; Hollings and Brunt, 1981; Shukla et al. 1991).

Serological and other evidence relating BlCMV and CABMV has been contradictory. Serological relationships of BlCMV or CABMV isolates to other potyviruses have been reported (Bock, 1973; Lima et al., 1979; Lovisolo and Conti, 1966; Uyemoto et al., 1973). Isolates of BlCMV and CABMV were also reported to be serologically related to BCMV (Behncken and Maleevsky, 1977; Lima et al., 1981; Lovisolo and Conti, 1966; Phatak, 1974; Uyemoto et al., 1973; Taiwo and Gonsalves, 1982; Purcifull and Gonsalves, 1985), and BlCMV was reportedly related to bean yellow mosaic virus (BYMV), soybean mosaic virus (SbMV), tobacco etch virus (TEV), watermelon mosaic virus 2 (WMV-2), dasheen mosaic virus (DsMV) and lettuce mosaic virus (LMV) (Lima et al., 1979). The CABMV isolate described by Bock (1973) showed no serological relationship to BYMV, soybean mosaic virus (SbMV), potato virus Y (PVY) and TEV. Similarly the Iranian isolate of CABMV showed no relationship with a Dutch isolate of BCMV or German isolates of BCMV or BYMV (Kaiser and Mossahebi, 1975).

An account of serological relationships reported for BlCMV and CABMV are summarized in Table 7.6.

In an attempt to compare BlCMV with CABMV isolates derived from infected seeds of *Vigna* pre-introductions and seed lots, our preliminary serological tests (DAS-ELISA) indicated that both BlCMV and CABMV were serologically distinct viruses. This study was expanded to verify the preliminary results and to investigate serological relationships between BlCMV and CABMV and with other potyviruses. For this purpose 20 isolates of BlCMV and 37 of CABMV derived from *Vigna* pre-introductions and seed lots were compared. A few isolates collected from infected field-grown cowpea plants were also included and BlCMV-Ga isolate and CABMV-Mor isolates were also included as reference isolates. These serological comparisons are herein reported.

MATERIALS AND METHODS

Virus isolates:

Twenty isolates of BlCMV and 37 of CABMV were derived from infected seeds of *Vigna* pre-introductions and seed lots from various sources. The Georgia isolate of BlCMV, BlCMV-Ga, was obtained from Dr. C. W. Kuhn, Department of Plant Pathology, University of Georgia, Athens, GA U.S.A., and CABMV isolate "Morocco" was kindly provided by Dr. D.

Gonsalves, Cornell University, Geneva, NY U.S.A. These two isolates served as reference isolates for each virus. All isolates were maintained in insect-free greenhouse conditions by successive mechanical transmission on susceptible cowpea genotypes. Fresh or desiccated infected leaf tissue containing each isolate was used to perform serological tests. The identity of each BlCMV and CABMV isolate was confirmed by DAS-ELISA with respective immunoglobulin G (IgG) and by electron microscopy (EM). The origin and details concerning each isolates tested in this study are presented in Appendix-2.

Serological tests:

Sources of polyclonal antiserum:

Polyclonal antiserum against a typical isolate of CABMV (RN-7C) was produced in our own laboratory by injecting purified virus to a New Zealand white rabbit, by procedures described in Chapter-3. Antiserum against BlCMV was provided by Dr. C. W. Kuhn.

Serological comparison with polyclonal antisera:

Serological comparisons with polyclonal antisera to BlCMV and CABMV were conducted by DAS-and DAC-ELISA, and Sodium dodecyl sulfate (SDS) immunodiffusion tests. DAS-ELISA was performed as outlined by Clark and Adamas (1977), while DAC-ELISA was carried out as described by Hampton

et al, (1992a). Procedure for DAS-and DAC-ELISA are described in chapter-3, Tables 3.2 & 3.5. Absorbance values (A_{405}) generated by ELISA reactions were recorded by measure of a BIO-TEK Automatic ELISA Reader Model E 109, 90 min after addition of enzyme substrate (p-nitrophenyl phosphate). Absorbance values for healthy-plant extracts were automatically subtracted from those of sap from infected plants.

Immunodiffusion tests were performed in agar plates prepared as described by Ball (1990). The gel medium consisted of 0.8% Noble Agar, 1.0% sodium azide and 0.5% SDS. Wells were cut in agar plates with a cork-borer of 6 mm diameter according to a pattern for one central and six peripheral wells. The central and peripheral wells were 5 mm from edge to edge. Antigens were prepared by grinding 0.1 g of fresh infected tissue in 1 ml extraction buffer (1 X PBS containing 1% SDS, pH 7.4).

Serological comparisons with monoclonal antibodies:

Potyvirus monoclonal antibodies of BCMV serotypes:

BlCMV and CABMV were compared by means of three monoclonal antibodies (MAbs), I-Z, II-197 and II-463 produced against BCMV epitopes and kindly provided by Dr. G. I. Mink, Irrigated Agriculture Research and Extension Center, Prosser WA, U.S.A. This group of MAbs distinguishes

epitopes characteristic of two serotypes A (I-Z) and serotype B (II-463) of BCMV. MAb II-197 reacts to an epitope shared by potyviruses against which it has been tested.

ELISA, antigen-coated plate (MAbs):

BlCMV and CABMV isolates were tested by indirect antigen-coated plate (ACP) ELISA (Jordan and Hammond, 1991). Antigens were prepared in antigen buffer (Carbonate coating buffer, CB; 0.05 M carbonate-bicarbonate, pH 9.6, containing 0.2% sodium diethyldithiocarbamate (DIECA) and 2% polyvinylpyrolindone (PVP) Mr 40.000). MAbs were cross-absorbed in healthy sap (500 X) for one hr at 37 C prior to loading wells. Dilutions of 3,000, 10,000 and 1000 of I-Z, II-197 and II-463 respectively were used, and anti-mouse globulin conjugate was used at a dilution of 1:1000.

RESULTS

Comparison of BlCMV and CABMV isolates by DAC and DAS-ELISA tests:

ELISA tests were performed with antisera/IgG to BlCMV and CABMV. Twenty *Vigna* seed-borne potyviral isolates reacted strongly with BlCMV antiserum in DAC or purified anti-BlCMV IgG in DAS-ELISA tests (Table 7.1). None of these 20 isolates reacted with CABMV antiserum or IgG in DAC or DAS-ELISA tests. All 20 potyviral isolates showed

Table 7.1: A_{405} values produced by extracts from cowpea tissue infected with BlCMV isolates, against antisera to BlCMV or CABMV in DAC and DAS-ELISA tests.

Isolates (antigens)	DAC-ELISA		DAS-ELISA	
	CABMV (AS)	BlCMV (AS)	CABMV (IgG)	BlCMV (IgG)
PI-3B	0.001[1]	1.231	0.010	1.105
RF-4B	0.002	1.473	0.001	1.401
PU-6B	0.010	0.894	0.002	1.464
PU-7B	0.002	0.984	0.012	0.987
PU-8B	0.002	1.024	0.003	2.287
PU-9B	0.001	1.115	0.031	1.248
PI-21B	0.020	1.079	0.002	1.025
PI-22B	0.001	1.328	0.004	1.011
PI-23B	0.023	2.116	0.000	1.597
PI-24B	0.012	1.732	0.002	0.758
PI-25B1	0.003	2.332	0.003	1.013
PI-25B2	0.005	2.217	0.017	1.027
PI-25B3	0.001	2.109	0.012	1.119
PI-25B4	0.021	1.987	0.041	0.997
RF-26B	0.001	0.992	0.021	1.110
RF-27B	0.004	1.320	0.020	0.908
PI-28B	0.002	1.119	0.004	1.731
PI-29B	0.012	2.201	0.001	1.003
COR-30B	0.017	1.117	0.002	1.710
Ga	0.031	2.114	0.007	2.060
Momologous (CABMV)	2.136	1.872	2.375	0.012
Homologous (BlCMV)	0.002	2.423	0.003	2.311
Healthy (cowpea)	0.004	0.012	0.014	0.007

[1] Values are average of two wells, recorded after 90 min incubation with substrate.
AS = Antiserum
IgG = Purified immunogammaglobulin.

serological homogeneity and were designated as BlCMV isolates.

In DAS-ELISA 35 potyviral isolates reacted strongly with CABMV IgG, whereas two isolates RN-27C and RN-28C reacted weakly (Table 7.2). None of the 37 CABMV isolates reacted with BlCMV IgG in DAS-ELISA. These 37 seed-borne potyviral isolates were designated as CABMV isolates. All CABMV isolates cross-reacted with BlCMV antiserum by DAC-ELISA, whereas BlCMV isolates did not cross-react with antiserum of CABMV. DAS-ELISA clearly partitioned 55 potyviral isolates into two distinct groups i.e. BlCMV and CABMV.

Relationship with other potyviruses:

Ten isolates, five each of BlCMV and CABMV were tested by DAC-ELISA against antisera to 10 potyviruses. A_{405} values produced 60 min after incubation of enzyme substrate at room temperature are shown in Table 7.3. None of the five BlCMV isolates reacted with antisera to BYMV, CABMV and WLMV, except that one isolate (PI-23B) reacted positively with BYMV antiserum. All five isolates of BlCMV reacted strongly with BCMV antiserum. In case of CYVV, PMV-1, PMV 204-1, PSbMV and TEV antisera a variability (no reaction to strong) in reaction of five BlCMV isolates was observed.

All CABMV isolates did not react with BYMV and WLMV antiserum except that one isolate (RN-7C) reacted

Table 7.2: A_{405} values produced by extracts from cowpea tissue infected with CABMV isolates, against antisera to BlCMV or CABMV in DAC and DAS-ELISA tests.

Isolates (antigens)	DAC-ELISA		DAS-ELISA	
	CABMV (AS)	BlCMV (AS)	CABMV (IgG)	BlCMV (IgG)
RN-2C	1.320	1.007	0.782	0.002
RN-4C	1.550	1.110	0.911	0.002
RN-6C	2.871	0.742	1.495	0.020
RN-7C	2.301	1.399	1.118	0.021
RN-10C	2.610	0.782	1.844	0.060
RN-11C	1.340	0.993	1.210	0.071
RN-12C	0.973	1.316	1.240	0.030
RN-13C	1.290	0.469	1.094	0.003
RN-14C	1.322	1.110	1.997	0.007
RN-15C	0.639	1.110	0.556	0.012
RN-18C	0.795	0.879	0.551	0.000
PI-23C	2.388	1.009	1.002	0.009
RN-27C	1.390	1.270	0.341	0.031
RN-28C	0.920	0.723	0.286	0.007
RN-29C	0.997	1.310	0.912	0.003
RN-30C	1.221	1.201	1.372	0.021
RN-31C	1.002	1.431	1.006	0.012
RN-32C	0.917	0.799	0.987	0.023
RN-33C	1.002	0.941	0.996	0.002
RN-34C	1.231	1.006	0.749	0.005
RN-35C	1.201	1.930	1.513	0.012
RN-36C	1.784	0.975	1.037	0.071
RN-37C	1.117	1.241	1.794	0.091
RN-38C	0.991	0.679	1.784	0.003
PI-39C	1.771	1.334	1.002	0.002
PI-40C	2.113	1.087	1.976	0.002
PI-41C	1.001	1.120	0.945	0.012
PI-42C	1.203	1.002	1.112	0.031
PI-43C	1.331	0.781	1.723	0.041
PI-44C	0.791	0.481	0.791	0.020
PK-1C	1.421	1.901	1.111	0.032
PK-2C	1.091	1.578	1.421	0.012
PK-5C	1.751	1.001	1.210	0.017
PK-6C	0.993	1.341	0.879	0.012
PK-10C	1.210	0.887	0.793	0.041

Cont,d....

Table 7.2: (Cont,d..)

Isolates (antigens)	DAC-ELISA		DAS-ELISA	
	CABMV (AS)	BlCMV (AS)	CABMV (IgG)	BlCMV (IgG)
CABMV (Bz)	2.345	1.105	2.712	0.012
CABMV (Mor)	2.109	1.189	2.110	0.004
Momologous (CABMV)	2.387	2.114	1.756	0.014
Homologous (BlCMV)	0.002	2.197	0.003	2.311
Healthy (cowpea)	0.014	0.011	0.018	0.003

[1] Values are average of two wells, recorded after 90 min incubation with substrate.
AS = Antiserum
IgG = Purified immunogammaglobulin.

Table 7.3: A_{405} values produced by extracts from cowpea tissue infected with BlCMV or CABMV isolates against antisera to potyviruses by DAC-ELISA.

Antisera	BlCMV isolates				CABMV isolates					
	Ga	10B	21B	23B	24B	7C	11C	23C	42C	Mor	Healthy
BlCMV	2.871	1.721	2.881	2.612	2.312	2.113	1.723	1.332	1.982	1.881	0.001
BCMV	1.650	1.202	2.332	2.292	0.893	1.137	0.179	0.210	0.218	0.219	0.004
BYMV	0.002	0.001	0.002	0.458	0.005	0.504	0.001	0.003	0.012	0.001	0.031
CABMV	0.004	0.005	0.012	0.045	0.009	2.411	2.911	2.110	1.978	1.720	0.021
CYVV	0.590	0.189	0.192	0.305	0.465	0.410	0.522	0.621	0.230	0.483	0.061
PMV-1 (204-1)	0.162	0.210	0.001	0.002	0.204	0.286	0.225	0.151	0.110	0.210	0.071
PMV	0.991	0.422	1.158	1.865	0.407	2.710	1.991	1.685	1.321	1.791	0.002
PSbMV	0.709	0.693	0.880	1.227	0.302	2.883	1.874	2.320	2.110	1.870	0.004
WLMV	0.001	0.065	0.075	0.045	0.071	0.001	0.021	0.004	0.005	0.004	0.061
TEV	0.007	1.322	1.360	2.971	0.635	0.442	0.645	0.477	0.535	0.616	0.003

†Values are average of two wells, recorded 60 min aftre incubation with substrate.

positively with BYMV antiserum in DAC-ELISA. In the case of antisera to other potyviruses there was a great variability in reaction depending upon isolate and virus antiserum. Among the five isolates of CABMV tested only RN-7C reacted strongly with BCMV antiserum. All five isolates reacted strongly with PMV 204-1 and PSbMV antisera, whereas the reactions with TEV, PMV-1 and CYVV were from weak to intermediate types.

Differentiation of BlCMV and CABMV isolates with MAbs:

Three MAbs, I-Z, II-197 and II-463 (Wang et al., 1984) were used to distinguish BlCMV from CABMV. Twenty isolates of BlCMV and 32 of CABMV were tested against these three MAbs by ACP-ELISA test. All isolates of BlCMV and CABMV did not react at all with MAb I-Z, whereas all isolates of BlCMV and CABMV reacted positively with MAb II-197 (Table 7.4). All CABMV isolates reacted with MAb II-197, but not with-463. MAb II-197 recognized all isolates of BlCMV as well as CABMV including other potyviruses. However, MAb II-463 was able to distinguish between BlCMV from CABMV. All 20 isolates of BlCMV and 32 of CABMV showed serological homogeneity within their serogroup.

Comparison of isolates in SDS-immunodiffusion tests:

Ten isolates of BlCMV and 14 of CABMV were tested by SDS-immunodiffusion tests. The reactions of BlCMV and CABMV isolates to homologous and heterologous antisera in

Table 7.4: Differentiation of BlCMV and CABMV isolates in antigen-coated plate (ACP) ELISA by use of monoclonal antibody (MAbs).

Isolates (antigens)	ACP-ELISA A_{405} values against MAbs.		
	I-Z	II-197	II-463
BlCMV isolates			
Ga	0.002[1]	2.581	1.482
PI-3B	0.001	1.032	1.102
RF-4B	0.012	1.338	0.981
PU-6B	0.002	1.087	1.312
PU-7B	0.017	2.789	1.270
PU-8B	0.023	2.176	1.013
PU-10B	0.011	2.216	1.300
PI-21B	0.016	2.897	1.006
PI-22B1	0.001	2.971	0.912
PI-22B2	0.003	1.672	1.333
PI-22B3	0.004	1.230	1.921
PI-23B	0.002	0.597	0.741
PI-24B	0.004	0.754	0.910
PI-25B1	0.002	2.322	1.110
PI-25B3	0.002	2.417	0.998
PI-25B4	0.020	1.987	1.987
PI-25B9	0.003	1.771	1.345
PI-26B	0.003	2.122	1.007
PI-27B	0.016	1.987	1.230
PI-28B	0.023	2.301	0.998
CABMV isolates			
RN-2C	0.012	2.037	0.009
RN-4C	0.012	2.649	0.034
RN-6C	0.006	0.974	0.007
RN-7C	0.043	1.923	0.001
RN-10C	0.034	1.340	0.005
RN-11C	0.012	1.112	0.070
RN-12C	0.030	0.981	0.010
RN-14C	0.004	1.257	0.004
PI-23C	0.004	1.777	0.009
RN-27C	0.045	1.024	0.002
RN-28C	0.067	0.942	0.023
RN-29C	0.005	1.111	0.002
PI-32C	0.005	1.200	0.023
RN-34C	0.006	0.989	0.002

Cont,d...

Table 7.4: (Cont,d..)

Isolates (antigens)	ACP-ELISA A_{405} values against 3 MAbs.		
	I-Z	II-197	II-463
RN-35C	0.051	1.007	0.008
RN-36C	0.007	1.009	0.007
RN-37C	0.021	1.853	0.045
RN-38C	0.006	1.001	0.009
PI-39C	0.007	0.998	0.003
PI-40C	0.004	1.432	0.097
PI-41C	0.007	1.042	0.087
PI-42C	0.002	2.341	0.054
PI-43C	0.023	2.110	0.001
PI-44C	0.045	1.865	0.007
PK-1C	0.012	1.234	0.045
PK-2C	0.005	1.087	0.001
PK-3C	0.009	2.145	0.007
PK-5C	0.032	0.677	0.003
PK-10C	0.007	1.121	0.008
PK-16C	0.002	1.222	0.010
CABMV-Bz	0.005	1.893	0.047
CABMV-Mor	0.009	1.245	0.081
BCMV (NL-2	0.873	0.676	2.128
BCMV (NL-3)	0.004	0.447	2.097
BCMV (NL-6)	0.646	0.474	1.750
BCMV (NL-8)	0.002	0.458	1.034
BCMV (NL-15)	0.001	0.449	0.678
BCMV (TS)	0.002	0.678	1.117
PSbMV[2]	0.002	1.551	0.012
WLMV	0.023	1.372	0.023
PMtV	0.010	1.078	0.003
CMV[3]	0.017	0.004	0.012
CSMV	0.001	0.003	0.017

[1]Values are average of two wells, recorded 60 min after incubation with substrate.

[2]PSbMV, WLMV and PMtV were included as potyviruses.

[3]CMV and CSMV were included as non-potyviruses.

SDS-immunodiffusion tests are shown in Table 7.5. Results of these tests agreed precisely with those from DAC-ELISA i.e. BlCMV isolates did not react (no band was produced) to antiserum of CABMV, whereas all the isolates of CABMV reacted (weak to strong) to antiserum of BlCMV, but bands formed by homologous antigens spurred over those by heterologous antigens, showing non-identity of BlCMV with CABMV isolates.

DISCUSSION

Fifty-five seed-borne (*Vigna*), geographically diverse potyviral isolates were compared serologically, including type isolates of BlCMV (BlCMV-Ga) and CABMV (RN-7C and CABMV-Mor) and their respective homologous antisera. These isolates probably had been associated with their respective native *Vigna* genotypes for multiple plant generations, and likely comprised representative, natural variants of each virus (i.e., free of experimentally induced artifacts). Preliminary results from DAS-ELISA partitioned the isolates into two distinct groups: 20 that reacted exclusively with anti-BlCMV IgG and 35 that reacted exclusively with anti-CABMV IgG. In subsequent multiple tests by DAS-ELISA, we had particular interest in the extent to which each isolate repeatedly fit within the two initial groups. The grouping was absolutely consistently in five successive tests,

Table 7.5: Reactions of BlCMV and CABMV isolates to homologous and heterologous antisera in SDS-immunodiffusion tests 36 hr after incubation.

Isolates (antigens)	Antisera to	
	CABMV	BlCMV
BlCMV isolates		
Ga	-	+
PU-7B	-	+
PU-8B	-	+
PI-21B	-	+
PI-23B	-	+
RF-26B	-	+
RF-27B	-	+
PI-24B	-	+
PI-25B1	-	+
PI-25B4	-	+
Healthy	-	-
CABMV isolates		
RN-4C	+	++*
RN-7C	+	++*
RN-14C	+	++*
PI-23C	+	+*
RN-27C	+	+*
RN-28C	+	+*
RN-34C	+	++*
RN-37C	+	++*
PI-39C	+	+*
PI-41C	+	+*
PI-43C	+	++*
PI-44C	+	+*
CABMV-Mor	+	++*
CABMV-Bz	+	+*
Healthy sap	-	-

\- = No reaction
\+ = Reaction with homology or identity
+* = Weak heterologous reaction, but homologous spurred over heterologous antigens
++* = Strong reaction, but homologous antigen spurred over heterologous antigens.

confirming the clear distinction by DAS-ELISA between isolates identified as either BlCMV or CABMV.

The same distinctions between isolates of the two viruses occurred with DAC-ELISA, using CABMV antiserum; distinctions that were not made by BlCMV antiserum. It is assumed that IgG's to antigenic determinants shared between the two viruses were abundant in BlCMV antiserum and lacking in CABMV antiserum. Reactions between seed-borne BlCMV or CABMV isolates and other well defined potyviruses indicated several shared antigenic determinants that probably did not represent true viral relationships. In essence, a finite number of antigenic determinants are shared among members of the potyvirus group, and thus serological reactions are more frequent than true relationships (Hollings and Brunt, 1981).

Patterns of precipitin-band spurring in immunodiffusion tests were consistent with the distinctions of isolates into two groups: those identified by DAS-ELISA, respectively, as either BlCMV or CABMV.

In this study four cowpea genotypes (TVU 401, TVU 1582, TVU 2657, and TVU 3433 were identified, which clearly distinguished 29/32 BlCMV isolates and 50/50 CABMV isolates, indicating that genetic resistance to each virus is conferred by distinct gene(s). There was only one exception that the BlCMV isolate: PU-7B, PU-8B, and PU-10B, which were derived from infected seed lots of Pusa Phalguni

(India), and were distinguished by DAS-ELISA, behaved like CABMV isolates on TVU-2657 and TVU-3433. Taiwo et al. (1982) also reported the usefulness of these TVU lines in distinguishing and separating in mixed infection these two viruses.

In contrast to Dijkstra et al. (1979) who reported that BlCMV and CABMV are not two distinct viruses, our serological and biological results clearly indicated that BlCMV and CABMV are two distinct potyviral entities. Dijkstra et al. (1979) limited their study to include only one laboratory isolate of CABMV to compare with four isolates of BlCMV. They probably ignored the importance of a large sample of seed-borne diverse isolates of BlCMV and CABMV for comparison.

Table 7.6: Serological relationship of BlCMV and CABMV to potyviruses, A-review of previous reports.

Virus	Serological test used	Relationships		Reference
		Related to	Unrelated to	
BlCMV	?	BCMV, WMM-2	BiMoV, ComMV, PeMtV, PRSV, PRSV, TuMV.	Uyemoto et al., 1973
BlCMV	Tube precipitation	BCMV	BYMV, PMV, Psbmv, CYVV, SbMV, PVY, TSEV, SCMV, IMV.	Bock, 1973
BlCMV	SDS-immunodi-ffusion	CABMV	-	Lima and Purcifull, 1979.
BlCMV	SDS-immuno-diffusion	BCMV PMV-1, BCMV-S, DMV, LMV, DYV, SbMV, TEV, WMV-2.		Lima et al., 1979
BlCMV	Drop precipitation	BYMV	BCMV	Mali and Kulthe, 1980[a]
BlCMV	?	BCMV, PStV		Purcifull & Gonsalves 1985. Demski et al., 1984
BlCMV	Slide agglutination	BYMV, BCMV, SbMV		Mali et al., 1988

Cont,d.

Table 7.6: (Cont,d..)

Virus	Serological test used	Relationships		Reference
		Related to	Unrelated to	
BlCMV	SDS-immuno-diffusion	BCMV	PVY, TuMV	Tsuchizkai, 1986
BlCMV	SDS-immuno-diffusion	BCMV	PVY, SbMV	Taiwo & Gonsalves, 1982.
BlCMV	SDS-immuno-diffusion	BYMV-Scott, PMV, CAMV(Mor), Pmtv, CYVV, PVY, Pstv, TEV, TuMV, WMV-2 ZYMV-Fl, ZYMV-RI		Zhao et al., 1991b
CABMV	?	BCMV, BYMV		Behncken & Maleevsky, 1977.
CABMV	?	BCMV		Lima et al., 1981
CABMV	?	BCMV		Lovisolo & Conti, 1974
CABMV	Micro-agglutination	BCMV	SbMV, PVY	Phatak, 1974
CABMV (Mor)	SDS-immnuno-diffusion		BCMV	Fischer and Lockhart, 1975.

Cont,d..

Table 7.6: (Cont,d..)

Virus	Serological test used	Relationships		Reference
		Related	Unrelated	
CABMV	Slide agglutination		BCMV, BYMV	Kaiser & Mossahebi, 1975.
CABMV	Slide agglutination	BCMV	BYMV, SbMV, PeMoV.	Mali et al., 1988
CABMV	SDS-immuno diffusion		PVY, TEV, SbMV	Taiwo & Gonsalves, 1982.
CABMV	SDS-immnu-nodiffusion	BlCMV	CYVV, IrMV,	Lima et al., 1978
			PSbMV	Lima et al., 1979
CABMV	?		BYMV	Heinze, 1957

BiMoV = Bidens mottle virus, ComMV = Commelina mosaic, PRSV = Papya ringspot virus SCMV = Sugarcane mosaic virus, IMV = Iris mosaic virus, SbMV = Soybean mosaic virus, PStV = Peanut stripe virus. BYMV = Bean yellow mosaic virus, BCMV = Bean common mosaic virus, TuMV = Turnip mosaic virus, PVY =Potato virus Y, TEV =Tobacco tech virus, PeMoV = Peanut mottle virus, WMV-2 = Watermelon mosaic virus, ZYMV = Zukuni yellow mosaic virus, DsMV = Dasheen mosaic virus.

CHAPTER-8

IDENTIFICATION, DISTRIBUTION, AND SEED TRANSMISSION OF COWPEA VIRUSES IN SENEGAL

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ABSTRACT

Viral diseases of cowpea in Senegal were surveyed during the rainy seasons of 1990 and 1991. ELISA tests using seven antisera of cowpea viruses with 66 virus-infected samples collected from the surveyed areas, indicated the presence of cowpea aphid-borne mosaic potyvirus (CABMV) (52%) alone or in combination with cowpea severe mosaic comovirus (CSMV), cowpea mottle carmovirus (CPMoV), and southern bean mosaic sobemovirus (SBMV). Two new potyvirus isolates seed-borne in cowpea and readily transmitted mechanically and non-persistently by *Aphis craccivora* Koch were identified. Both isolates, detected by DAC-ELISA using potyvirus-selective monoclonal antibody II-197 (Wang et al., 1984) were partially characterized and were unrelated to BlCMV or CABMV potyviruses. The two

isolates were differentiated from each other by their consistent weak (V-1) and strong (V17-14) reactions against potyvirus monoclonal antibodies and by their reactions in 18 selected plant species and genotypes. The unnamed potyvirus isolates occurred principally in new, improved CABMV-resistant cowpea genotypes. CABMV and these potyvirus isolates were the most prevalent viruses in the sampled cowpea-growing Districts of Senegal.

In tests of 35 cowpea cultivars/lines, seven genotypes, TVU 401, TVU 402P2, TVU 410, TVU 1000, TVU 1016-1, TVU 1582, and White Acre-BVR were resistant to both potyvirus isolates. These genotypes are therefore available for breeding resistant cowpea cultivars.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is the second most important legume crop grown in Senegal after groundnut and is annually grown on some 63,000 hectares, with an annual production of 18,000,000 kg. The average cowpea yield in Senegal, 280 kg/ha, is 14% of 2000 kg/ha yields from Senegal experimental plots. (Hall, 1991).

Among cowpea viruses reported in West Africa (Raheja and Leleji, 1974; Williams, 1975; Lana and Adegbola, 1977; Thottappilly & Rossel, 1985; Mali & Thottappilly, 1986), cowpea yellow mosaic comovirus (CPYMV) and cowpea aphid-

borne mosaic potyvirus (CABMV) are economically most important. Other viruses known to occur in West Africa are cowpea mottle carmovirus (CPMoV) and southern bean mosaic sobemovirus (SBMV) (Lamptey & Hamilton, 1974; Thottappilly & Rossel, 1985; Gaikwad & Thottappilly, 1987). Cowpea mild mottle carlavirus (CMMV), cowpea severe mosaic comovirus (CSMV), and cucumber mosaic cucumovirus (CMV) had also been detected in seed lots from Burkina Fasso, Nigeria and Senegal, and Ghana respectively (Hampton et al, 1992).

Seed-borne viruses are considered a major constraint on yield in farm fields (Gaikwad, 1988), because emerging plants are quickly exposed to viral inoculum producing greater damage at early stages of crop plant development. Two recently developed lines, IS86-275 N and IS86-283-15 N were expected to contribute to the increase and sustainability of cowpea production, because of their resistance to bacterial blight (*Xanthomonas campestris* pv *vignicola*), CABMV, storage weevil (*Bruchus spp.*), striga (*Striga gesnerioides*) and drought (Hall, 1991). Infections of these lines in field trials, however, suggested the occurrence of indigenous unidentified viruses or unidentified pathotypes of CABMV in Senegal. The present study was conducted to identify these viruses, to evaluate their importance as pathogens, and identify resistant cowpea genotypes for use in developing virus resistant cultivars.

MATERIALS AND METHODS

Field survey and virus identification:

During the rainy seasons of 1990 and 1991 fields were surveyed for viral diseases in the five major cowpea production areas in Senegal. A total of 66 samples were collected from plants showing virus-like symptoms in 37 farm fields and station trials. The samples were desiccated over $CaCl_2$ for subsequent study and reference. The samples were then tested by enzyme-linked immunosorbent assay (ELISA) for possible presence of seven cowpea viruses in the Virology Laboratory, Department of Botany and Plant Pathology, Oregon State University , U.S.A., blackeye cowpea mosaic (BlCMV) and cowpea aphid-borne mosaic (CABMV) potyviruses, cowpea severe mosaic (CSMV) and cowpea mosaic (CPMV) comoviruses, cowpea mottle carmovirus (CPMoV), cucumber mosaic cucumovirus (CMV), and southern bean mosaic sobemovirus (SBMV). The samples were also tested against two potyvirus-selective monoclonal antibodies, Agdia PVY and II-197 (Wang et al., 1984). Either double antibody sandwich (DAS) ELISA (Clark and Adams, 1977) or direct antigen coating (DAC) ELISA (Hampton et al, 1992a) were used for the tests.

Disease reactions, seed transmission and host range tests:

Preliminary evaluations for the disease reactions of advanced breeding lines to four virus isolates derived from

field collected samples (V-1, and V-2, collected from naturally infected field grown cowpea plants in Kolda and V-17 and V-54 from Diourbel) were carried out both under screenhouse and field conditions. Five of the most advanced cowpea cultivars/lines (Table 8.2) of cowpea grown in Senegal were mechanically inoculated with tissue extracts from diseased plants, after dusting with carborundum powder. The plants were inoculated again three days later to minimize 'escapes'. Disease observations were recorded biweekly from 7 to 45 days after inoculation. Disease incidence was calculated as the percentage of inoculated plants that became infected. Insecticide was applied as needed to control insects.

Seed lots from five cowpea cultivars (Table 8.2) were collected from naturally infected field-grown plants as well as from mechanically inoculated plants. Both were tested for seed-borne viruses by growing seedlings in sterilized soil in insect-free glasshouses. Seedling infection was determined by symptoms on primary and first trifoliate leaves. Asymptomatic and selected symptomatic plants were tested by DAC-ELISA using potyvirus monoclonal antibody II-197.

Virus isolates were derived from seed-borne infections of single plants of cowpea cultivars "58-57" and "Mougne" that had been mechanically inoculated under screenhouse conditions. Stock cultures were maintained on susceptible

cowpea cultivars. A range of plant species and genotypes were tested for susceptibility to five seed-borne potyvirus isolates,V1-1, V17-2, V17-14, V54-3 and V54-23. The tests were conducted in insect free glasshouse conditions, where temperature (28-30 C) and light conditions (14 hr photoperiod; 125-175 langleys) were provided for optimum cowpea growth and development. Eight to ten plants of each host species (Table 8.3) were inoculated at stages pre-determined for optimal susceptibility. Five weeks after inoculation, symptomless plants were assayed by DAC-ELISA using potyvirus monoclonal antibody.

Serological relationships:

All virus isolates derived from infected seeds of cowpea cvs 58-57, Mougne, Baye Ngagne, IS86- 275N and IS86-283N were tested by DAS-ELISA against immunoglobulin G (IgG) to BlCMV, CABMV, CMV and pea seed-borne mosaic virus (PSbMV), and by DAC-ELISA against antisera of the following potyviruses: BlCMV, CABMV, clover yellow vein virus (CYVV), peanut mottle virus (PeMoV), peanut stripe virus (PStV), pea seed-borne mosaic virus (PSbMV), white lupin mosaic virus (WLMV) as well as antibodies Agdia PTY-1 potyvirus-selective monoclonal and II-197. The purity of the unknown potyviruses were also tested in DAC-ELISA against antisera to cowpea mottle carmovirus (CPMoV), CPMV, CSMV and SBMV. Absorbance values were recorded by a BIO-TEK Automatic ELISA reader Model EL-309, 90 hours after

addition of substrate (p-nitrophenyl phosphate). Viral extracts were prepared either from fresh or desiccated tissues from infected plants.

Electron microscopy:

A Philips Electron Microscope (EM) Model CM 12 was used to visualized virions of V17-14 potyvirus isolate from crude extracts of infected leaves or from purified preparation after adsorption to carbon coated copper grids, and negatively staining with 2% ammonium molybdate (pH 7.0).

Aphid transmission:

Aphid transmissibility (*Aphis craccivora* Koch) of potyviruses V1-1 and V17-14 was tested using aphids reared on healthy cowpea plants under controlled conditions. Plant-to-plant transmission was carried out as follows: after a 2 hr fasting period, groups of aphids were deposited on detached virus-infected cowpea leaves for a 3 to 4 min acquisition period. Those found in probing position were carefully transferred to healthy plants of cowpea cultivar 58-57. Twenty-seven to 35 test plants were inoculated with each isolate, using three aphids per plant. Aphids were allowed to feed overnight on test plants before removal by aerosol insecticide. Inoculated plants were observed for symptoms development for four weeks after exposure to aphids. Symptomless plants were assayed by ELISA. Each transmission test was repeated three times.

Identification of resistant sources:

An expanded group of cowpea genotypes were subsequently tested (Table 8.6) for two reasons: (a) to identify potential differential cultivars, for distinguishing PTY+ isolates from other potyviruses seed-borne in cowpea, and (b) to identify sources of resistance to these isolates, to support practical control efforts through resistance breeding. For this purpose, 35 genotypes were inoculated in the glasshouse with each of five PTY+ isolates.

RESULTS

Field survey and virus identification:

Of 66 samples collected from five cowpea growing areas of Senegal, 59 reacted positively with either one or more of the seven test antisera or monoclonal antibodies (Table 8.1). Thirty-four of 66 samples were found to contain CABMV either alone or combined with CSMV, CPMoV or SBMV. CABMV was predominant at all of the locations surveyed. One sample from Diourbel contained both CSMV and CPMoV. SBMV was detected in only one sample from Louga, in mixture with CPMoV. Neither BlCMV, CMV, nor CPMV was detected among the test samples. Twenty-one samples reacted with potyvirus monoclonal antibody II-197, but reacted with no other test IgG or antiserum. Based on these reactions and

Table 8.1: Cowpea viruses detected in field collected samples of cowpea in Senegal (West Africa).

Locations surveyed	Samples collected (no.)	Number of samples reacted positively with antiviral antisera/potyvirus MAbs, when tested in DAC-ELISA.							
		BlCMV	CABMV	CMV	CPMV	CPMoV	SBMV	SBMV	MAb
Diurbel	28	-	10	-	-	1	1	-	11
Kolda	4	-	1	-	-	-	-	-	2
Louga	20	-	10	-	-	1	-	1	7
Tamba	10	-	9	-	-	-	-	-	1
Ties 4	4	-	4	-	-	-	-	-	-
Total:	66	-	34	-	-	2	1	1	21

- : No virus detectable by ELISA
MAb: Monoclobal antibody (II-197)

supplementary serological and hosts range test, the isolates V1 and V17 were temporarily designated as potyvirus PTY+. One (V17) reacted strongly with II-197, and with PTY-1 potyvirus monoclonal antibodies, while the other (V1) reacted weakly with either monoclonal antibody (Table 8.3). These potyvirus isolates and CABMV were the most prevalent cowpea viruses at all locations surveyed.

Disease reaction, seed transmission, and host range tests:

Results of field inoculations (Senegal) of five selected CABMV-resistant cowpea genotypes with four sources of potyvirus PTY+, and subsequent seed-transmission results, are presented in Table 8.2. With some variation in numbers of plants infected by the five isolates, all genotypes were susceptible to this distinct potyvirus. Symptoms induced under glasshouse conditions were essentially the same as those in inoculated, field-grown plants of the selected genotypes. Rates of seed-transmission among isolates and among cowpea genotypes ranged from 0% to 32%. Numbers of available seeds were too small, however, to assess detailed differences for these interactions.

The PTY+ isolates were subjected to a limited non-Vigna host range test, for comparison with published data for other potyviruses. For this purpose, 13 standardized host genotypes were inoculated with the five seed-borne isolates (Table 8.3). Symptoms induced by the fiveisolates

Table 8.2: Disease incidence[1] and seed transmission[2] (seed from inoculated, diseased plants). of potyvirus isolates.

Virus isolate	Cultivars/ lines	Disease incidence (%)	No of seeds germinated/ planted	Incidence transmission (%)
V 1	Baye Ngagne	100	37/50	1/37 (3)[3]
	IS86-275N	61	41/50	0/41 (0)
	IS86-283-15	8	29/50	0/29 (0)
	Mougne	97	46/50	2/46 (4)
	58-57	100	46/50	6/46 (12)
V 2	Baye Ngagne	100	46/100	0/46 (0)
	IS86-275N	47	37/100	0/37 (0)
	IS86-283-15	18	44/100	0/44 (0)
	Mougne	100	92/100	1/92 (1)
	58-57	55	83/100	7/83 (8)
V 17	Baye Ngagne	81	35/50	0/35 (0)
	IS86-275N	50	40/50	2/40 (5)
	IS86-283-15	52	23/50	0/23 (0)
	Mougne	97	47/50	0/47 (0)
	58-57	93	43/50	13/43 (32)
V 54	Baye Ngagne	86	27/50	0/27 (0)
	IS86-275N	88	40/50	2/40 (5)
	IS86-283-15	82	28/50	0/28 (0)
	Mougne	97	49/50	0/49 (0)
	58-57	89	44/50	1/44 (2)

1: Experiment conducted under field conditions.
2: Experiment conducted under greenhouse conditions.
3: Figures in paranthesis are percent seed-transmission.

Table 8.3: Limited host range of five potyvirus isolates.

Host species	Disease reactions of potyvirus isolates				
	V1-1	V17-2	V17-14	V54-3	V54-23
A. Leguminous hosts:					
Lupinus luteus cv Astra	-	-	-	-	-
Medicago sativa cv DuPuits	-	-	-	-	-
Trifolium pratense cv Kenland	-	-	-	-	-
Phaseolus vulgaris cv Monroe	-	-	-	-	-
Phaseolus vulgaris cv Top Cross	-	-	LI	-	-
Vicia faba cv Hertz Fveya	VN	VN	VN	VN	VN
B. Non-leguminous hosts:					
Chenopodium amaranticolor cv. Corvallis sstrain	LLn	LLn	LLn.VN	LLn	LLN,VN
Nicotiana benthamiana	SM	SM	SM	SM	SM
Gomphorena globosa A.F. Ross Strain	-	-	-	-	-
Phlox drumondii cv Tall Mixed color	-	-	-	-	-
Lycoperson esculentum cv Marglobe	-	-	-	-	-
Petunia hybrida cv King Henry	-	-	-	-	-
Antirrhinum majus L. cv Mixed Colors	-	-	-	-	-

- = No symptoms and no detectable virus by ELISA
LLn= Necrotic local lesions
VN= Vein necrosis
SM= Systemic mosaic

were essentially the same, except that two of them, V17-14 and V54-3, induced vein necrosis in addition to necrotic local lesions on inoculated leaves of *Chenopodium amaranticolor*. In essence, isolates exhibited a very narrow host range. For instance, CABMV (a possible relative) infects numerous *P. vulgaris* cvs, *M. sativa* cv DuPuits, and *G. globosa*, capacities apparently lacking in the PTY+ isolates.

Serological relationships:

Five seed-borne PTY+ isolates were tested by DAS-ELISA against selected potyviruses (relationships) and CMV (contamination) and by DAC-ELISA to a larger group of potyviruses (possible relationships) and other seed-borne non-potyviruses (contamination) (Table 8.4). In DAS-ELISA, the PTY+ isolates were confirmed to be unrelated to BlCMV or CABMV, and also did not react to anti-PSbMV IgG. By DAC-ELISA, however, the PTY+ isolates reacted in varying degrees with antisera to six selected potyviruses (BlCMV, CABMV, PeMoV, PMV, PStV, and PSbMV), while not reacting to CYVV or WLMV. Such reactions are not assumed to indicate relationships with PTY+ isolates. The PTY+ isolates were not contaminated with ELISA-detectable concentrations of CMV, CPMoV, CSMV, or SBMV.

Electron microscopy:

Plants infected with potyvirus isolates (V1-1 and V17-14) contained flexuous rod-shaped particles when viewed as

Table 8.4: Serological comparison of five seed-borne potyvirus isolates in cowpea with other poty and non-potyviruses by DAS and DAC ELISA tests.

Antiserum	A_{405} values 90 min after incubation with substrate						
	Seed-borne potyvirus isolates					Virus homologous	Healty sap
	V1-1	V17-2	V17-14	V54-3	V54-23		
A. DAS-ELISA							
BlCMV	0.001	0.005	0.014	0.001	0.009	0.947	0.005
CABMV	0.011	0.013	0.005	0.014	0.030	1.109	0.013
PSbMV	0.001	0.003	o.001	0.004	0.003	1.871	0.002
CMV	0.006	0.002	0.008	0.001	0.002	1.270	0.019
B. DAC-ELISA							
BlCMV	0.256	1.200	0.358	0.366	0.207	2.812	0.016
CABMV	1.680	1.649	1.512	1.302	1.405	2.476	0.023
CYVV	0.105	0.098	0.078	0.052	0.077	>3.00	0.010
PeMoV	1.630	1.375	2.027	1.265	1.580	>3.00	0.030
PStV	1.902	1.500	1.751	1.096	1.918	>3.00	0.018
PMV	1.862	0.540	0.231	0.775	0.505	>3.00	0.016
PSbMV	1.652	1.578	1.514	1.855	2.108	>3.00	0.008
WLMV	0.012	0.022	0.014	0.023	0.043	>3.00	0.005
CSMV	0.156	0.103	0.113	0.154	0.127	>3.00	0.123
SBMV	0.004	0.004	0.002	0.002	0.017	>3.00	0.011
BMMV	0.027	0.024	0.023	0.024	0.003	>3.00	0.034
P.MAb (II-197)	0.307	0.771	1.400	0.573	0.700	0.582[a]	0.001
Agdia (P.MAb)	0.220	NT	0.697	0.781	0.855	0.769[b]	0.002

NT: Not tested a: BlCMV (RF-26B isolate) b: CABMV (9-7C isolate)

leaf dip or purified preparations. The modal length of the particles was found to be 725 nm (Figure 8), which fits within the recognized size range of potyvirus particles (710 - 900 nm).

Aphid transmission:

Seed-borne PTY+ isolates V1-1 and V17-14 were tested for non-persistent transmissibility by *Aphis craccivora* Koch. Apterous aphids that had been fasted for 2 hr were placed in groups of 15 to 20, on detached leaves from infected cowpea plants. Aphids that had remained in feeding position for 3 to 4 min were carefully moved to healthy '58-57' cowpea test plants, three aphids per plant. In separate experiments, isolate V1-1 was transmitted to 42 of 61 (69.4%) inoculated plants and isolate V17-14 was transmitted to 59 of 59 (100%) inoculated plants (Table 8.5).

Identification of resistant sources:

The reactions of cowpea cultivars/lines to mechanical inoculation of five potyvirus isolates, V1-1, V17-2, V17-14, V54-3 and V54-23 are shown in Table 8.6. Of 34 test cultivars/lines , seven IITA-TVU lines, TVU 401, TVU 402 P2, TVU 410, TVU 1000, TVU 1016-1 and TVU 1582 and one U.S. promising cultivar were found to be immune to all potyvirus isolates tested. Resistant sources to individual isolates were also identified.

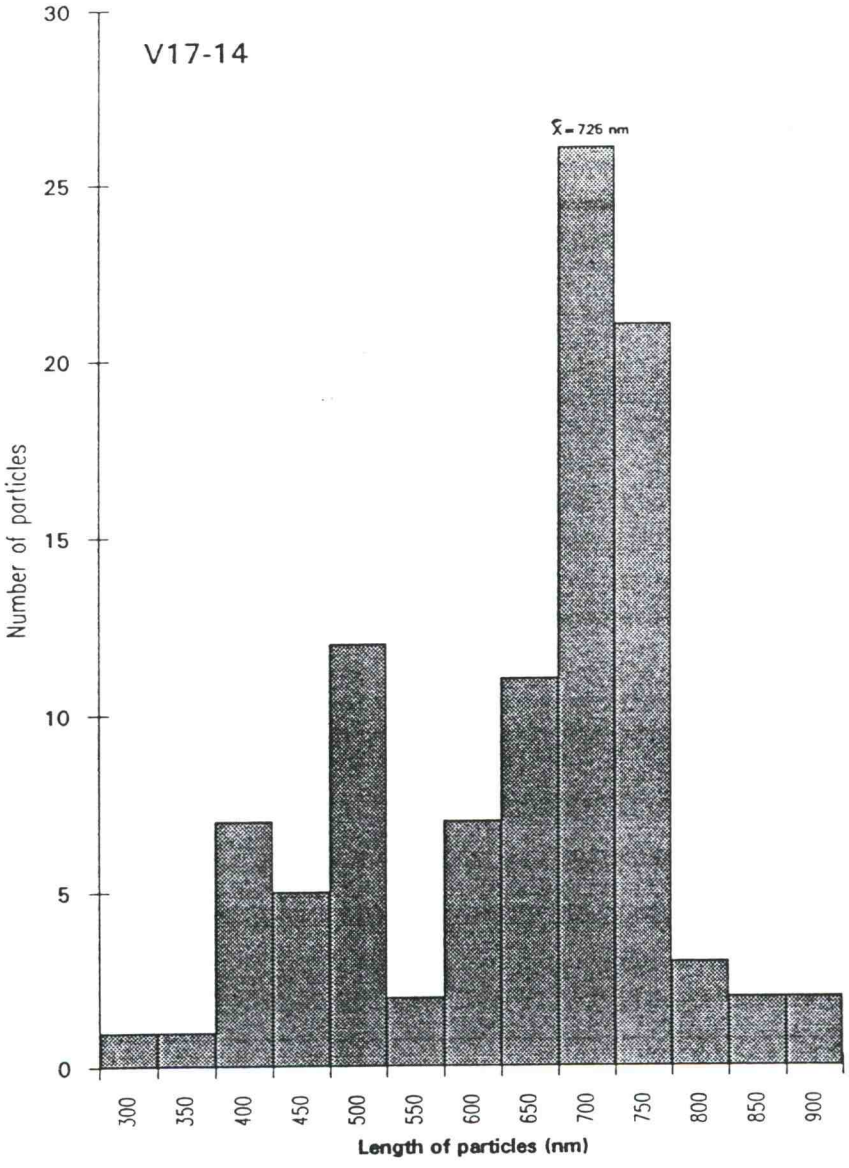

Figure 8. Histogram of V17-14 potyvirus particle lengths, partially purified virus preparation. The modal length of particles was 725 nm.

Table 8.5: Tramsmission of two potyvirus isolates V1-1 and V17-14 by *Aphis craccivora* with feeding on infected cowpea plants cv 58-57 to healthy cowpea plant.

Isolate	Experiment	Percent transmission		Average (%)
V1-1	1	19[1]/26	$(73.0)_2$	69.4 a
	2	23/35	(65.7)	
V17-14	1	28/28	(100)	100.0 b
	2	31/31	(100)	

[1]Numbers infected/per numbers tested; three aphids per plant.

[2]Treatments followed by different letter (a,b) in the same column differ significantly at P=0.05 level, according to Duncton's Multiple Range test.

Table 8.6: Response of cowpea genotypes to manual inoculations with five seed-borne potyvirus isolates under greenhouse conditions.

Genotypes	Potyvirus isolates				
	V1-1	V17-2	V17-14	V54-3	V54-23
TVU 109P2	-	++	++	-	++
TVU 196	++	-	++	++	++
TVU 347	-	++	-	++	-
TVU 354	++	++	++	++	++
TVU 401	-	-	-	-	-
TVU 408P2	-	-	-	-	-
TVU 410	-	-	-	-	-
TVU 984	++	++	++	++	-
TVU 1000	-	-	-	-	-
TVU 1016-1	-	-	-	-	-
TVU 1582	-	-	-	-	-
TVU 2657	++	++	++	++	++
TVU 3433	++	++	++	++	++
IT 81D 1137	-	++	++	++	++
IT 86 27N	++	++	++	++	++
PI 25122	++	++	++	++	++
Bambey 21	LI	-	_	++	++
Serido	-	-	++	-	-
W.A.BVR	-	-	-	-	-
CBE 5	0	0	+	++	0
Snapre	0	0	+	++	0
Blue Goose	0	0	-	-	0
Corona	0	0	-	-	0
Mopod	0	0	-	-	0
T. C.# 8	0	0	++	++	0
T. C.# 40	0	0	-	++	0
UCR 524B	0	0	-	-	0
M. Purple	0	0	++	++	0
M. Silver	0	0	++	++	0
Magnolia	0	0	++	-	0
K.P.Hull	0	0	-	-	0
Worthmore	0	0	++	++	0
Bettergreen	0	0	++	++	0
UCR 7964	0	0	++	++	0

- = No symptoms, virus nor detectable by ELISA
++= Moderate systemic infection, with visible symptoms
0 = No symptom, virus not detectable by ELISA, immune
TVU genotyupes were receieved from I.I.T.A. Ibadan, Nigeria.

DISCUSSION

Cowpea viruses are becoming more important in all cowpea growing areas of Senegal. The survey reported herein was prompted because new cultivars/lines had become damaged by virus diseases. These cultivars/lines had been developed specifically for resistance to CABMV. Seed-borne viruses were designated as priority pathogens, since they inflict heavy losses through primary field inoculum followed by secondary insect-vector spread (Kaiser and Mossahebi, 1975).

The present study of 66 Senegal cowpea samples with virus-like symptoms indicated the presence of four viruses, CABMV, CSMV, CPMoV, and SBMV naturally infecting cowpeas. SBMV had already been reported from the Casamance region of Senegal (Gaikwad and Thottappilly, 1987). Hampton et al., (1992a) detected CSMV in germplasm accession from Senegal.

Our results indicated that CABMV was the most prevalent virus (52% of the test samples) in all of the locations surveyed. An unnamed potyvirus, PTY+, was second in occurrence (21/66 = 32%) to CABMV. Occurrence of CABMV, SBMV, and CPMoV in naturally infected cowpea plants is believed to be the first report in Senegal. SBMV was detected only in one sample from Louga in mixture with CPMoV. Multiple virus infection in field- grown plants modify symptoms and essentially preclude field diagnosis

(Kuhn et al.,1966). CPMoV was previously reported only from Nigeria (Robertson, 1966, Rossel, 1977, Shoyinka et al., 1978), and was recently reported from Pakistan (Bashir and Hampton, 1991). CPMoV was also detected from cowpea samples collected from screening nurseries in Riverside, California, U.S.A (M. Bashir, unpublished results). The seed-borne nature of CPMoV (Allen et al.,1982) and recent detections suggest that the virus could be spreading through seeds to other parts of the world. CMV and CPMV were expected to occur in Senegal samples, but were detected in none of the 66 samples.

Two new potyvirus isolates, V1-1 and V17-14 were partially characterized. The two isolates differed serologically, but only minor differences were found in their biological properties. Isolate V1-1 reacted very weakly to potyvirus monoclonal antibody II-197 and Agdia PVY MAb, while V17-14 reacted strongly to both. Isolate V1-1 produced necrotic local lesions on inoculated leaves of *C. amaranticolor*, whereas V17-14 induced necrotic local lesions as well as vein necrosis. Both isolates resembled CABMV in their failure to infect cowpea cultivars, TVU-401 and TVU-1582, which proved resistant to all tested CABMV isolates (Bashir and Hampton, 1992).

In DAS-ELISA both PTY+ isolates reacted negatively with IgGs of CABMV and BlCMV, indicating serological non-relatedness. Reactions of PTY+ isolates to BlCMV and

CABMV antisera and the antisera to PSbMV, PeMoV, and MStV were indications of shared epitopes among potyviruses, not necessarily relatedness. The two PTY+ isolates were found by DAC-ELISA to be free of CSMV, CPMV, CPMoV, and SBMV.

While various measures such as control of insect vector, rouging of diseased plants and production of virus-free seed may reduce the field incidence of viral diseases in cowpea, the development of resistant cultivars is viewed as the most economical and practical method for controlling viral diseases of cowpea. In this study, we identified at least seven cowpea genotypes, TVU-401, TVU-402P2, TVU-410, TVU-1000, TVU-1016-1, TVU-1582, and White Acre BVR, as sources of resistance to the new potyvirus isolates. We therefore, propose the use of these genotypes for developing resistant cultivars adapted to Senegal cowpea production areas.

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APPENDICES

APPENDIX-1

LIST OF VIGNA PRE-INTRODUCTION AND GERMPLASM ACCESSIONS EVALUATED FOR DETECTION OF SEED-BORNE POTYVIRUSES.

S.No.	Accession No.	Cultivar	Country	Seed status
1	PI 186454	-	Nigeria	Increased
2	PI 186458	-	"	"
3	PI 186459	-	"	"
4	PI 186460	-	"	"
5	PI 186465	-	"	"
6	PI 186466	-	"	"
7	PI 186467	-	"	"
8	PI 194202	-	U.S.A Maryland	Original
9	PI 194203	-	"	Increased
10	PI 194204	-	"	"
11	PI 194205	-	"	"
12	PI 194206	-	"	"
13	PI 194207	-	"	"
14	PI 194208	-	"	"
15	PI 194209	-	"	"
16	PI 194210	-	"	"
17	PI 194211	-	"	"
18	PI 211109	-	Afghanistan	"
19	PI 211110	-	"	Original
20	PI 211111	-	"	Increased
21	PI 211641	-	"	"
22	PI 211642	-	"	"
23	PI 211753	-	"	"
24	PI 211754	-	"	"
25	PI 211755	-	"	"
26	PI 211756	-	"	"
27	PI 212635	-	"	"
28	PI 214069	-	India	Original
29	PI 214353	-	"	"
30	PI 214354	-	"	"
31	PI 218123	Lobia	Pakistan	Increased
32	PI 220847	Lobia	Afghanistan	"
33	PI 220848	-	"	"
34	PI 220849	-	"	"
35	PI 220850	-	"	"
36	PI 220851	-	"	"
37	PI 221729	-	South Africa	"
38	PI 221730	-	"	"

Cont,d..

Appendix-1 (Cont,d..)

S.No.	Accession No.	Cultivar	Country	Seed status
39	PI 221731	-	South Africa	Increased
40	PI 221732	-	"	Original
41	PI 222755	Lobia	Iran	Increased
42	PI 222756	Lobia	Iran	Increased
43	PI 223023	Lobia	"	"
44	PI 223420	Lobia	"	"
46	PI 227397	Lobia	"	"
47	PI 229551	-	"	"
48	PI 229734	Chesh	"	"
49	PI 229735	Lobia	"	"
50	PI 229796	-	"	"
51	PI 250416	-	Pakistan	"
52	PI 255760	Ushikic	Nigeria	"
53	PI 255765	_	"	"
54	PI 255766	-	"	"
55	PI 256342	-	Pakistan	"
56	PI 269664	-	India	"
57	PI 269666	Pusa	"	"
		Barsali	"	"
58	PI 270068	-	"	"
59	PI 271256	-	"	"
60	PI 271257	-	"	"
61	PI 271258	-	"	"
62	PI 271259	-	"	"
63	PI 292889	-	South Africa	"
64	PI 292890	-	"	Original
65	PI 292891	-	"	"
66	PI 292892	-	"	"
67	PI 292893	Bechuana	"	Increased
		White	"	"
68	PI 292896	-	Hungary	"
69	PI 292897	-	"	Original
70	PI 292898	-	"	"
71	PI 292899	-	"	"
72	PI 292900	-	"	Increased
73	PI 292901	-	"	Original
74	PI 292902	-	"	Increased
75	PI 292903	-	"	Original
76	PI 292904	-	"	Increased
77	PI 292905	-	"	Original
78	PI 292912	-	South Africa	Increased
79	PI 302457	-	U.S.A	"

Cont,d..

Appendix-1 (Cont,d..)

S.No.	Accession No.	Cultivar	Country	Seed status
80	PI 302458	-	U.S.A	Increased
81	PI 307554	M. Favorite	"	"
82	PI 307556	FC 31660	"	"
83	PI 307557	Browneye	"	"
		Simpson	"	"
84	PI 307558	FC 31738	"	"
85	PI 307559	FC 31739	"	"
86	PI 307560	FC 31740	"	"
87	PI 307561	Purple Hull	"	"
88	PI 312202	-	Mexico	Original
89	PI 312203	-	"	"
90	PI 312204	-	"	"
91	PI 312206	-	"	"
92	PI 312206	-	Mexico	Original
93	PI 312208	-	"	Increased
94	PI 312210	-	"	Original
95	PI 312211	-	"	"
96	PI 352830	IC 180	India	Increased
97	PI 352832	IC 323	"	"
98	PI 352833	-	"	"
99	PI 352834	IC 389	"	"
100	PI 352835	IC 394	"	"
101	PI 352836	IC 409	"	"
102	PI 352837	IC 434	"	"
103	PI 352838	IC 435	"	"
104	PI 352839	-	"	"
105	PI 426229	-	Afghanistan	"
106	PI 426230	-	"	"
107	PI 426231	-	"	"
108	PI 426232	-	"	"
109	PI 4262	-	"	"
110	PI 419101	ssp.	China	"
		V. U. sesquipedalis		"
111	PI 419102	"	"	"
112	PI 419163	"	"	"
113	PI 419164	"	"	Original
114	PI 419165	"	"	"
115	PI 419166	"	"	"
116	PI 419167	"	"	Increased
117	PI 419168	"	"	"
118	PI 419169	"	"	"
119	PI 427065	-	Pakistan	Increased

Contd,..

Appendix-1 (Cont,d..)

S.No.	Accession No.	Cultivar	Country	Seed status
120	PI 427066	-	Pakistan	Increased
121	PI 427068	-	"	"
122	PI 427070	-	"	"
123	PI 427071	-	"	"
124	PI 427072	-	"	"
125	PI 427073	-	"	"
126	PI 427075	-	"	"
127	PI 427076	-	"	"
128	PI 427078	-	"	"
129	PI 427080	-	"	"
130	PI 427081	-	"	"
131	PI 441917	-	Brazil	"
132	PI 441924	-	"	"
133	PI 441925	-	"	Original
134	PI 447430	-	Nigeria	Increased
135	PI 447431	-	"	"
136	PI 471440	-	Iran	Original
137	PI 471441	-	"	"
138	PI 471442	-	"	"
139	PI 471443	-	"	"
140	PI 471444	-	"	"
141	PI 471445	-	"	"
142	PI 471446	-	"	"
143	PI 471447	-	"	"
144	PI 471448	-	"	"
145	PI 471449	-	"	"
146	PI 487554	-	Brazil	Increased
147	PI 491450	-	Botswana	Original
148	PI 491451	-	"	"
149	PI 491452	-	"	"
150	PI 491454	-	"	"
151	PI 491455	-	"	"
152	PI 491456	-	"	"
153	PI 491457	-	"	"
154	PI 491458		"	"
155	PI 491459	-	"	"
156	PI 353003	-	India	Original
157	PI 218123	-	Pakistan	"
158	PI 251222	-	Afghanistan	"
159	Pak 45261	-	Pakistan	"
160	Pak 45262	-	"	"
161	Pak 45262	-	"	"

Contd,..

Appendix-1 (Cont,d..)

S.No.	Accession No.	Cultivar	Country	Seed status
162	Pak 45264	-	Pakistan	Original
163	Pak 45439	-	"	"
164	Pak 45440	-	"	"
165	Pak 45441	-	"	"
166	Pak 45445	-	"	"
167	Pak 45447	-	"	"
168	Pak 45449	-	"	"
169	Pak 45443	-	"	"
170	Pak 27001	-	"	"
171	Pak 27002	-	"	"
172	Pak 27004	-	"	"
173	Pak 27005	-	"	"
174	Pak 27007	-	"	"
175	Pak 27008	-	"	"
176	Pak 29009	-	"	"
177	Pak 27010	-	"	"
178	Pak 27011	-	"	"
179	Pak 27012	-	"	"
180	Pak 27013	-	"	"
181	Pak 27014	-	"	"
182	Pak 27015	-	"	"

APPENDIX-2

LIST OF BlCMV AND CABMV POTYVIRUS ISOLATES DERIVED FROM GERMPLASM ACCESSIONS, SEED LOTS, OR FIELD GROWN PLANTS.

S.NO.	Isolate	Cultivar/line/ accession No.	Origin of cultivar/line accession No. or seed lots	Location of isolate or seed source
A. <u>BlCMV isolates</u>.				
1	PI-3B	PI 353303	India	PIC, GA.
2	RF-4B	CBE-5	California	Poplar plot Riverside, California.
3	PU-6B	Pusa Phalguni	India	The seed of Pusa Phalguni was obtained from Dr.P.N. Patel, California.
4	PU-7B	" "	"	
5	PU-8B	" "	"	
6	PU-9B	" "	"	
7	PU-10B	" "	"	-
8	PU-11B	" "	"	-
9	PU-12B	" "	"	-
10	PU-13B	" "	"	-
11	PU-14B	" "	"	-
12	PU-15B	" "	"	-
13	PU-16B	" "	"	-
14	PU-17B	" "	"	-
15	PU-18B	" "	"	-
16	PU-19B	" "	"	-

Cont,d..

Appendix-2 (Cont,d..)

S.NO.	Isolate	Cultivar/line/ accession No.	Origin of cultivar/line accession No. or seed lots	Location of isolate or seed source
17	PU-20B	Pusa Phalguni	India	P.N.Patel
18	PI-21B	PI 352830	India	PIC, GA.
19	PI-22B1	PI 487554	Brazil	"
20	PI-22B2	"	"	"
21	PI-22B3	"	"	"
22	PI-22B4	"	"	"
23	PI-22B5	"	"	"
24	PI-22B6	"	"	"
25	PI-22B7	"	"	"
26	PI-23B	PI 269664	India	"
27	PI-24B	PI 292902	Hungary	PIC. GA.
28	PI-25B1	PI 352839	India	"
29	PI-25B2	"	"	"
30	PI-25B3	"	"	"
31	PI-25B4	"	"	"
32	PI-25B5	"	"	"
33	PI-25B6	"	"	"
34	PI-25B7	"	"	"
35	PI-25B8	"	"	"
36	PI-25B9	"	"	"

Contd,..

Appendix-2 (Cont,d..)

S.NO.	Isolate	Cultivar/line/ accession No.	Origin of cultivar/line accession No. or seed lots	Location of isolate or seed source
37	RF-26B	Unknown	California	Riverside, California.
38	FR-27B	Unknown	"	"
39	PI-28B	PI 251222	Afghanistan	Seed was increased in California.
40	PI-29B	"	"	"
41	COR-30B	Cornet	?	?
42	COR-31B	"	"	"
43	BlCM-Ga) (Represents type isolate)		-	This isolate was supplied by Dr.C.W. Kuhn, GA. U.S.A
B. <u>CABMV isolates</u>.				
1	RN-2C	878-1	Botswana	Riverside nursery, California.
2.	RN-3C	878-2	"	
3	RN-4C	876-1	"	"
4	RN-6C	876-3	"	"
5	RN-7C	876-4	"	"
6	RN-10C	876-7	"	"
7	RN-11C	1177-1	"	"
8	RN-12C	1177-2	"	"
9	RN-13C	1177-3	"	"
10	RN-14C	1177-5	"	"

Contd,..

Appendix-2 (Cont,d..)

S.NO.	Isolate	Cultivar/line/ accession No.	Origin of cultivar/line accession No. or seed lots.	Location of isolate or seed source
11	RN-15C	1177-6	Botswana	Riverside nursery
12	RN-18C	1177-7	"	California.
13	PI-23C	PI 491454	"	PIC, GA.
14	RN-27C	58-57	Senegal	Riverside California.
15	RN-28C	U-449	Botswana	"
16	RN-29C	UCR 2571	"	"
17	RN-30C	UCR 1956	"	"
18	RN-31C	UCR 1867	"	"
19	PI-32C	PI 218123	Pakistan	The seed was obtained from P.N.Patel California.
20	PI-33C	PI 218123	"	"
21	RN-34C	UCR 1148	Botswana	California.
22	RN-35C	UCR 1179-1	Botswana	Riverside nursery,
23	RN-36C	UCR 1179-2	"	California.
24	RN-37C	UCR 1141	"	"
25	RN-38	UCR 850	"	"
26	PI-39C	PI 491450	Botswana	PIC, GA.
27	PI-40C	PI 491454	Botswana	"
28	PI-41C	PI 223720	India	"
29	PI-42C	PI 218123	Pakistan	"

Cont,d..

Appendix-2 (Cont,d..)

S.NO.	Isolate	Cultivar/line/ accession No.	Origin of cultivar/line accession No. or seed lots.	Location in case of field isolate.
30	PI-43C	PI 220847	Afghanistan	PIC, GA
31	PI-44C	PI 302458	U.S.A	PIC, GA
32	CA-45C	?	?	?
33	PK-1C	Local	Pakistan	-
34	PK-2C	"	"	-
35	PK-3C	"	"	-
36	PK-4C	"	"	-
37	Pk-5C	"	"	-
38	PK-6C	"	"	-
39	PK-7C	"	"	-
40	PK-8C	"	"	-
41	PK-9C	"	"	-
42	PK-10C	"	"	-
43	PK-11C	"	"	-
44	PK-12C	"	"	-
45	PK-13C	"	"	-
46	PK-14C	"	"	-
47	PK-15C	"	"	-
48	PK-16C	"	"	-

Cont,d..

Appendix-2 (Cont,d..)

S.NO.	Isolate	Cultivar/line/ accession No.	Origin of cultivar/line accession No. or seed lots.	Location in case of field isolate.
49	PK-17C	"	Pakistan	-
50	PK-18C	"	"	-
51	PK-19C	"	"	
52	PK-20C	Local	"	-
53	PK-22C	"	"	-
54	CABMV-Bz		-	Supplied by Dr.C.W. Kuhn, GA.
55	CABMV-Mor (Represents type isolate)		-	Supplied by Dr.G Gonsalves.