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Sources of genetic resistance in cowpea (*Vigna unguiculata* (L.) Walp.) to cowpea aphid-borne mosaic potyvirus

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Abstract

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

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important high protein food and fodder crop of many tropical and subtropical countries of the world. Among the factors involved in censing yield losses in cowpea, some virus diseases are most important. Cowpea is reported to be infected by more than 20 viruses under field and experimental conditions (Thottappilly and Rosell, 1985). Among these cowpea aphid-borne mosaic (CABMV) is the most widespread in cowpea crops. The nature and severity of symptoms induced by CABMV vary with host cultivar, virus isolate, and time of infection. Yield losses of 13 to 87 per cent have been reported from Iran (Kaiser and Mossahebi, 1975). Although reasonable virus disease control can be achieved through several methods, host plant resistance is likely the most economical and practical approach to control CABMV. Sources of CABMV resistance have been reported (Ladipo and Allen, 1979; Taiwo et al., 1982; Mali et al., 1988) and are being used in cowpea improvement. In the present paper, we report some new cowpea genotypes immune, resistant and tolerant to seven CABMV isolates of diverse geographical origin.

Materials and methods

Virus isolate

Six isolates of CABMV were recovered from infected seed of cowpea germplasm obtained from the Plant Introduction Centre, Athens, Georgia, USA. One Moroccan representative isolate of CABMV was obtained from Dr. G. Gonsalves, Department of Plant Pathology, Cornell University, Geneva, USA, and included in this study (Table 1). The identity of each isolate was confirmed by using Agdia potyvirus monoclonal antibody and polyclonal antiserum to CABMV by enzyme-linked immunosorbent assay (ELISA), SDS-immunodiffusion assay and host reactions (Bashir, 1992). Each isolate was obtained through single lesion isolation from *Chenopodium amaranticolor* Coste et Reyn., and maintained in susceptible cowpea cultivar by serial mechanical transfer. The virus-infected plants were grown in insect-free greenhouses and used as inoculum source for the inoculation tests. Precautions were taken to avoid isolate mixing.

Source of cowpea genotypes

Fifty-one cowpea genotypes were obtained from the following sources: six TVu lines from the International Institute of Tropical Agriculture (IITA), Ibadan,

Table 1. Source of CABMV isolates used in this study

Isolate	Accession no.	Origin of accession	Source of seed
RN-4C	878-1	Botswana, Africa	Dr. P. N. Patel University of California, USA.
RN-27C	58-57	Senegal, Africa	" " " "
PI-32C	PI 218123	Pakistan	Plant Introduction Centre, Athens, Georgia, USA.
PI-41C	PI 223720	India	" " " "
PI-43C	PI 220847	Afghanistan	" " " "
PI-44C	PI 302458	USA	" " " "
CABMV-Mor	–	Morocco	Dr. G. Gonsalves, Cornell University, USA.

Nigeria, 23 promising U.S. cowpea cultivars from Dr. O. L. Chambliss, Department of Horticulture, Auburn University, Alabama, USA, and 22 advanced lines from Dr. T. E. Hall, University of California, Riverside, USA. Most cultivars/lines were multiplied under insect-free greenhouse conditions to ensure freedom from seed-borne diseases and to produce sufficient seed for experimental purposes.

Screening method

Twelve to sixteen seeds of each genotype were grown in sterilized soil in plastic pots of 23 cm diameter. Ten plants (5 plants/pot, 2 pots/genotype) of each genotype were maintained for mechanical inoculation. Seven to eight days old, fully expanded primary leaves of the seedlings of each genotype were separately sap inoculated with each virus isolate according to the standard procedure. Virus-infected leaves (1 g/10 ml) were triturated by mortar and pestle in 0.02 M phosphate buffer, pH 7.0 to prepare the inocula. Each inoculum was applied on cowpea leaves dusted with carborundum powder (600 mesh). To check the inoculum effectiveness the same number of plants of a susceptible cowpea variety were inoculated each time with the same inoculum. Buffer inoculated plants served as negative control. Two weeks after the inoculation, all the virus-inoculated plants not showing symptoms were reinoculated to avoid any escape. They were then kept under observation in insect-free greenhouses for at least six weeks. The reactions on the inoculated plants were recorded at two weeks interval. The primary leaves inoculated were observed for local symptoms, and the whole plants for systemic reaction. Mixed leaf tissue samples from the symptomless plants of each treatment were tested two times at monthly intervals by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) to distinguish immunity from symptomless infection (tolerance). The genotype which showed no symptoms and were negative in ELISA were tested three times to confirm their immunity.

Results

Each isolate induced a wide range of symptoms in plants of the susceptible genotypes. The characteristic symptoms of CABMV were observed in genotypes that reacted systemically with the individual isolates. They consisted of vein-banding, interveinal chlorosis, and distortion, blistering, and stunting of the leaves (Figures 1 and 2). In general, four types of isolate-genotype interactions were observed: *immunity*, in which inoculated plants remained symptomless and the virus was not detected by ELISA; *hypersensitivity*, characterized by the development of necrotic local lesions not followed by systemic spread of virus; *tolerance*, in which systemic infection occurred without the appearance of symptoms; and *susceptibility*, in which visible systemic symptoms appeared. The isolate CABMV-Mor (the representative isolate of CABMV) induced necrotic lesions in inoculated plants of PI 218123 followed by whole plant necrosis called lethal susceptibility (Figure 3). In some genotype delayed symptoms (ds) were

Figure 1. Mild (left) and severe (right) systemic symptoms induced by CABMV isolate RN-27C in cowpea genotypes PI 218123 and 58-57 respectively.

expressed. These genotypes remained symptomless for 2–3 weeks, then developed mild to severe systemic symptoms.

The reaction induced by the various virus isolates in both inoculated leaves and whole plants of each cowpea genotype are presented in Table 2. Of 51 genotype tested, TVu-401, TVu-1582, and TVu-1593 were found immune to all the seven CABMV isolates, whereas, there other genotypes, namely Pusa Phalguni, PI 218123, and PI 251222, were susceptible to all virus isolates, except that the isolate CABMV-Mor induced lethal necrosis in PI 2181123 within two weeks after inoculation. The remaining 45 genotypes were susceptible to CABMV, reacting with different symptoms to the various virus isolates.

The genotypes found immune, resistant and tolerant to individual isolates are listed in Table 3. Immunity was more common than resistance and tolerance. Among the isolates CABMV-Mor had a wider range of pathogenicity than other isolates. Only six genotypes were found to be immune to this isolate, whereas from 14 to 24 cowpea genotypes were immune to other isolates. Two types of resistance was observed among the genotypes. One was hypersensitive response (HR) not followed by systemic spread of virus, and the other was mild to moderate systemic infection. In case of CABMV-Mor and PI-43C isolates, none of the genotypes showed HR, whereas the other isolates induced HR in one or more than one genotypes (Table 2).

Discussion

The interaction between the seven CABMV isolates of geographically diverse origin (Afghanistan, Botswana, India, Pakistan, Senegal, and USA) and 51 cowpea genotypes resulted in four distinct reactions: immune, resistant, tolerant, and susceptible. Only two isolates CABMV-Mor and PI-44C induced lethal susceptibility (LS) in some genotypes (Table 2). In this case the death of the whole plants occurred within two weeks after inoculation. Such reaction by CABMV isolates has probably not been reported before. Both local lesion reactions with no systemic infection and LS reactions

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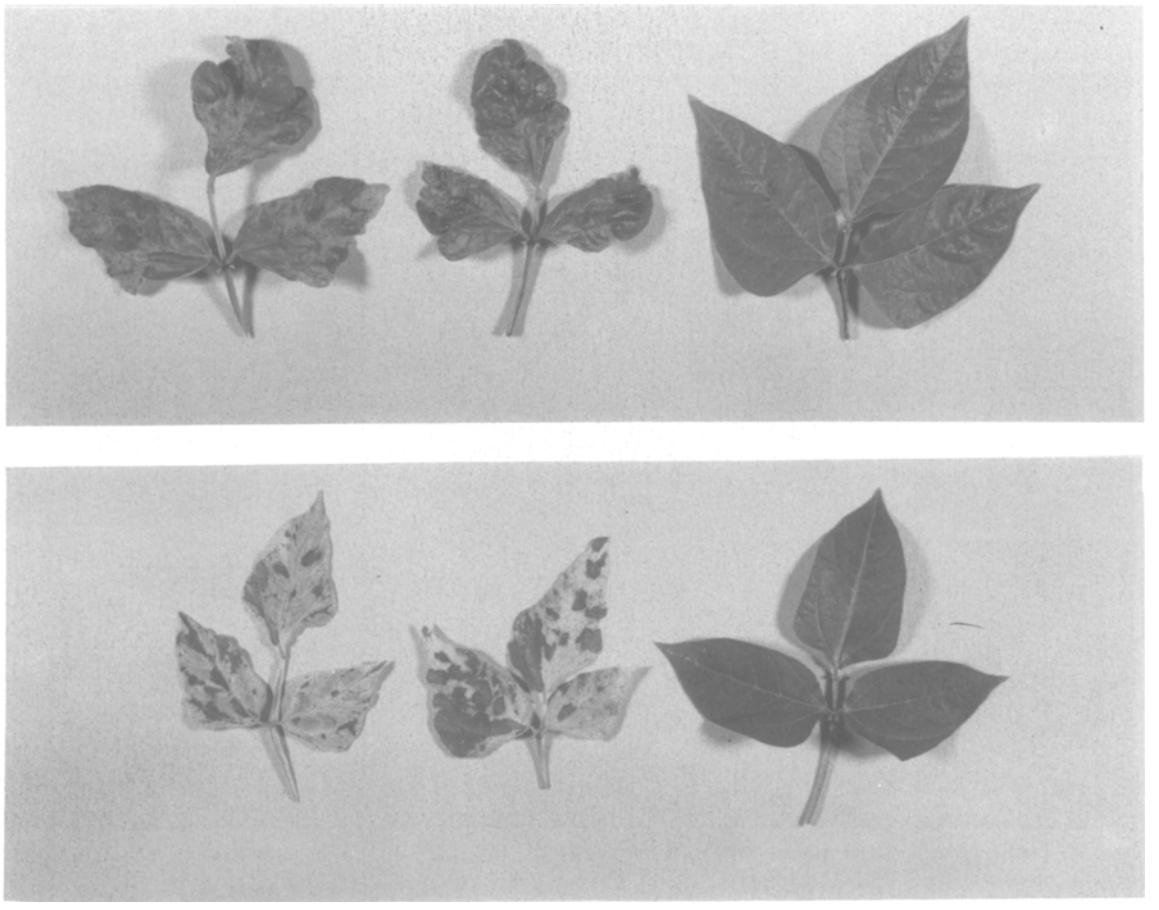

Figure 2. Variability in symptoms (left) induced by CABMV isolates: (a) CABMV-Mor (b) PI-32C (c) RN-27C and (d) PI-44C induced in the same cowpea genotype PI 251222. Healthy control on the right.

are considered hypersensitive response to the CABMV isolates (Patel and Kuwite, 1982).

Three lines TVu-2657, TVu-2740, and TVu-2845 characterized by delayed symptoms (ds) did not show symptoms for about two to three weeks, but subsequently developed clear mosaic of moderate to mild intensity. Some such lines could easily be rated as immune or resistant if the observation were not extended beyond four weeks after inoculation. Similar observations in the same lines were also reported by Patel et al. (1982) against an isolate of CABMV from Tanzania.

In contrast to the results reported by Patel et al. (1982), who found that TVu-401, TVu-1582, and TVu-1593 were susceptible to an isolate of CABMV from Tanzania, we found that these lines were immune to all the isolates tested in this study. The immunity of these TVu lines has previously been reported by Ladipo and Allen (1979) against an isolate of CABMV from Nigeria and by Taiwo (1978) against CABMV isolate from USA.

In our results immunity was more common than resistance and tolerance, and this contradicts with the findings of Kaiser and Mossahebi (1975), who failed to locate immunity among 1054 cowpea lines tested against an Iranian isolate of CABMV. However, they found several lines resistant to CABMV. Ladipo and Allen (1979) reported 52 lines immune, and 6 tolerant (symptomless virus carrier) to a Nigerian isolate of CABMV. Similar levels of host resistance to CABMV have been reported by other workers (Patel et al., 1982; Mali et al., 1988).

Some of the released US-cowpea cultivars were found either immune, resistant or tolerant to some of the isolates tested. For example, Purple Hull Pinkeye BVR, White Acre BVR, Bettergreen, and Corona were immune to all isolates except CABMV-Mor. Similarly, Serodo, and Knucle Purple Hull and CBE-3

Figure 2. Continued.

were immune to four isolates. The resistance/immunity of these cultivars has been reported against blackeye cowpea mosaic virus (BlCMV) and other cowpea viruses such as cowpea chlorotic mottle virus, cucumber mosaic virus, cowpea mosaic virus and southern bean mosaic virus (Collins et al., 1985). These cultivars possess multiple resistance and therefore must be included in breeding virus resistance high yielding varieties.

The cowpea genotypes; TVu-109P2, TVu-347, and TVu-1000, which are reported susceptible to a US CABMV isolate by Taiwo (1978) proved immune to five of seven isolates used in this study. Some of the genotypes gave distinct differential reactions against some CABMV isolates (Table 2). This indicates that CABMV isolates vary pathogenically. Bock (1973) also reported pathogenic variation in this virus. The differential reactions of several cowpea genotypes included in the tests of Taiwo (1978), Patel and Kuwite (1982) and Patel et al. (1982), and this study clearly indicates the existence of distinct pathogenic variation of CABMV. Although more isolates are required to test to determine the extent of pathogenic variation within the virus, this study suggests that sources of broad spectrum resistance to CABMV isolates are available for breeding purpose.

Table 2. Reaction of cowpea genotypes to mechanical inoculations of CABMV isolates

Genotype	Virus isolates													
	CABMV-Mor		RN-27C		RN-4C		PI-32C		PI-41C		PI-43C		PI-44C	
	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	·	–	·	–	·	–	·	–	·	–	·	–	·	–
TVu 2657	·	+++	·	+ds	·	–	·	+++	·	+ds	·	+++	·	+++
TVu 2740	·	+++	·	+ds	·	–	·	+++	·	+ds	·	Li	·	+++
TVu 2845	·	+++	·	+ds	·	+++	·	+++	·	+ds	·	+++	·	+++
TVu 3433	·	+++	·	+++	·	–	·	+++	·	+++	·	Li	·	+++
IT 80S 2049	·	+++	○	○	·	+++	·	+++	·	+++	·	+++	·	+++
CBE 3	·	+++	·	–	·	–	·	–	·	Li	·	–	·	–
CBE 5	·	+++	·	+++	·	+++	·	+++	NL	++	·	++	·	+++
CBE 46	·	+++	·	+++	·	+++	·	+++	NL	Li	·	–	·	–
CBE 88	·	+++	·	+++	·	++	·	+++	NL	++	·	–	·	+++
UCR 7964	·	+++	·	+++	·	+ds	·	+++	NL	++	·	–	·	+ds
UCR 8517	·	–	·	+++	·	+ds	·	+++	NL	–	·	–	·	+++
UCR 8679	·	+++	·	+++	·	–	·	–	·	++	·	–	·	–
UCR 160	·	+++	·	+++	·	+++	·	+++	·	+++	·	+++	·	+++
UCR 1393	·	+++	·	+++	·	+++	·	+++	·	+++	·	+++	·	+++
UCR 524B	·	+++	·	+++	·	+++	NL	+++	·	+++	·	++	·	+++
P.P.H.BVR	·	+++	·	–	·	–	·	–	·	–	·	–	·	–
White.BVR	·	+++	·	–	·	–	·	–	·	–	·	–	·	–
Serodo	·	+++	·	–	·	–	·	–	·	–	·	–	NL,V	–
Bettergreen	·	+++	·	–	·	–	·	–	·	–	·	–	NL,V	–
M. Purple	·	+++	·	+++	·	Li	·	Li	·	+++	·	Li	NL,N	++
M. Silver	·	+++	·	+++	○	○	○	○	·	Li	○	○	NL,V	++
Mopod	·	+++	·	–	·	++	·	–	·	–	○	○	○	○
Corona	·	+++	·	–	·	–	·	–	·	–	○	○	·	–
Worthmore	·	+++	·	+++	·	Li	·	Li	·	+++	○	○	NL,V	–
T.W. Crowder	·	+++	·	+++	·	–	·	–	·	+++	○	○	·	–
T.Cream #8	·	+++	·	–	○	○	·	–	·	–	○	○	·	++
T.Cream #40	·	+++	·	–	·	–	NL	–	NL	–	○	○	·	–
Blue Goose	·	+++	·	–	·	–	·	–	·	++	○	○	○	○
M.Shipper	·	Li	·	+++	·	Li	NL,V	Li	·	++	○	○	NL	+++
Snappea	·	+++	○	○	○	○	○	○	·	++	○	○	○	○
Big Boy	·	+++	·	–	·	–	·	–	·	–	○	○	·	–
B.S.Crowder	·	+++	·	+++	·	–	·	++	·	+++	·	+++	NL	+++
M. Cream	·	+++	·	+++	·	–	·	–	·	+++	○	○	·	Li
Magnolia	·	+++	·	+++	·	Li	NL	–	NL	+++	○	○	NL	+++
K.P. Hull	·	+++	·	–	·	Li	NL	–	·	–	·	–	·	–
T. Pinkeye 196	·	+++	·	–	·	–	NL	–	·	–	·	–	·	–
Pusa Phalguni	·	+++	·	+++	·	+++	·	+++	·	+++	·	+++	·	+++
PI 218123	·	+++	·	+++	·	+++	·	+++	·	+++	·	+++	·	+++
PI 251222	NL,V	Ls	·	+++	·	+++	·	+++	·	+++	·	+++	·	+++

· = No hypersensitive response (no local lesion induction); ○ = Not tested; – = Immune, no visible symptoms and no virus detection by ELISA; + = Very mild systemic mosaic; ++ = Moderate type systemic mosaic, not much severe; +++ = Very severe disease symptoms (highly susceptible); Li = Latent infection, no visible symptoms, virus detected by ELISA; NL = Necrotic local lesion (hypersensitive response); V = Vein necrosis on the inoculated primary leaves. Ls = Lethal susceptible, the plants were killed completely within two week after inoculation; Sy = Systemic infection; ds: delayed symptoms (symptoms appeared 2 to 3 weeks after inoculation).

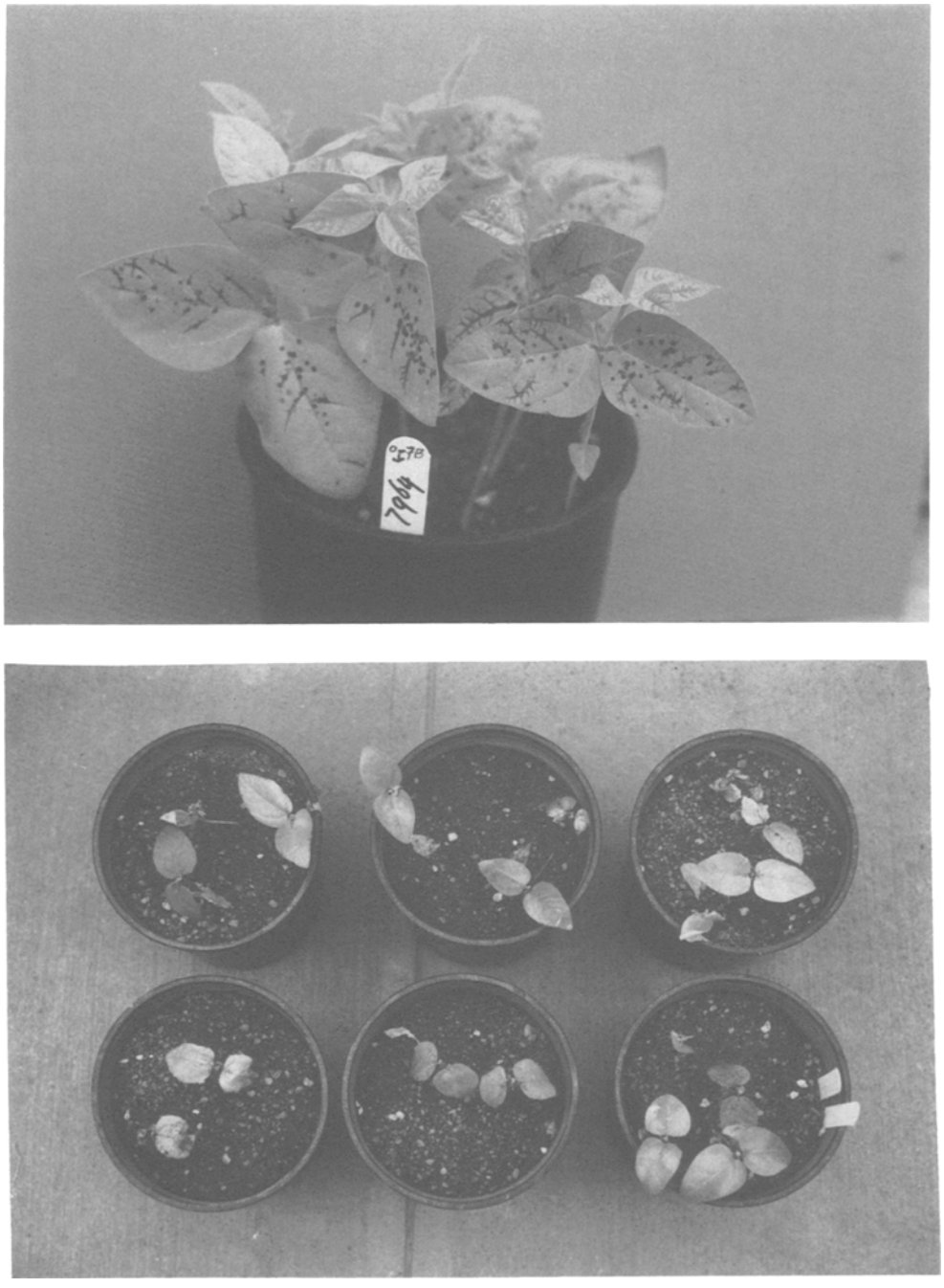

Figure 3. (top) Hypersensitive response with necrotic local lesions and vein necrosis in the inoculated leaves induced by CABMV isolate PI-41C in cowpea genotype UCR-7964 (bottom) Lethal susceptibility induced by CABMV-Mor isolate in cowpea genotype PI 318123 within two weeks after inoculation.

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Table 3. Cowpea genotypes immune, resistant and tolerant to individual CABMV isolates

Isolate	Genotypes		
	Immune	Resistant	Tolerant
CABMV-Mor	TVu-401, TVu-408P2, TVu-410, TVu-1582, TVu-1593, UCR-8517	–	M. Shipper
RN-27C	TVu-410, TVu-1000, TVu-1016-1, TVu-1693 CBE-3, P.P.H.BVR, White Acre BVR, Serodo, Bettergreen, Mopod, Corona, T.Cream No. 8, T.Cream No. 40, Big Boy, K.P. Hull, T. Pinkeye	–	–
RN-4C	TVu-401, TVu-109P2, TVu-347, TVu-1000, TVu 1016-1, TVu-1582, TVu-1593, TVu-2657, TVu-2740, TVu-3433, CBE-3, UCR-8679, P.H.P. BVR, White Acre BVR, Serodo, Bettergreen, Corona, T.W. Crowder, T.Cream No. 40, Blue Goose, Bog Boy, B.S. Crowder, M. Cream, T. Pinkeye.	TVu-408P2, TVu-410	M. Purple, Worthmore M. Shipper Magnolia Knuckle Purple
PI-32C	TVu-109P2, TVu-347, TVu-401, TVu-1000, TVu-1016-1, TVu-1582, TVu-1593, CBE-3 UCR-8679, P.P.H. BVR, White Acre BVR, Serodo, Bettergreen, Mopod, Corona, T.W. Crowder, T.Cream No. 8, T.Cream No. 40, K.P. Hull, T. Pinkeye	TVu-408P2, TVu-410, T. Cream No. 40 Magnolia, K.P. Hull, T. Pinkeye	CBE-3, CBE-46, M. Silver
PI-41C	TVu-109P2, TVu-347, TVu-401, TVu-408P2, TVu-410, TVu-1000, TVu-1016-1, TVu-1582 TVu-1593, UCR-851, P.P.H. BVR, White Acre BVR, Serodo, Bettergreen, T.Cream No. 8, T.Cream No. 40, Big Boy, K.P.Hull, T. Pinkeye	TVu-408P2, TVu-410, M. Silver CBE-5, CBE-88, T.Cream No. 40	CBE-3, CBE-46,
PI-43C	TVu-109P2, TVu-347, TVu-410, TVu-408P2, TV-410, TVu-984, TVu-1000, TVu-1016-1, TVu-1582, TVu-1593, IT 80S 2049, CBE-3, CBE-46, CBE-88, UCR 7964, UCR 8517 UCR-8079, P.P.H.BVR, White Acre BVR, Serodo, Bettergreen, K.H. BVR, T. Pinkeye	–	TVu-2657, TVu-2845 M. Purple
PI-44C	TVu-109P2, TVu-401, TVu-408P2, TVu-1582, TVu-1593, CBE-3, UCR-8679, P.P.H. BVR, White Acre BVR, Corona, T.W. Crowder, Big Boy, K.P. Hull, T. Pinkeye	TVu-984, UCR-7964 Serodo, Bettergreen, M. Purple, M. Silver, Worthmore	TVu-347 TVu-410 M. Cream

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