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SCREENING COWPEA (*VIGNA UNGUICULATA* (L) WALP) LINES FOR INFECTION RESPONSES TO SOME COWPEA VIRUSES IN NIGERIA.

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ABSTRACT

Thirty-three cowpea varieties were screened in a screen-house in Ibadan, Nigeria for responses to four cowpea viruses; namely, Bean common mosaic *potyvirus* - blackeye cowpea strain (BCMV-BIC), Cowpea aphid-borne mosaic *potyvirus* (CABMV), Cowpea Mottle *Carmovirus* (CMeV) and Southern bean mosaic *sobemovirus* (SBMV). 33 x 5 factorial experiment in completely randomised design was used, disease severity was measured at the second and sixth weeks after *mechanical* inoculation of the viruses and scored from 1 (no infection) to 5 (very severe infection). Symptomless plants were serologically tested with Protein – A sandwich enzyme linked immunosorbent assay (PAS-ELISA) to distinguish lines with latent infection from none infected lines. The yield in 2000 was not significantly ($p < 0.05$) different from the yield in 1999, similarly, the symptoms expressed in 1999 were not significantly ($p < 0.05$) different from those expressed in 2000. Nine cowpea lines were severely or very severely infected by the four viruses. Three viruses; BCMV-BIC, CABMV and SBMV did not infect two lines (IT90k-284-2 and IT82D-889). In addition, CABMV and BCMV-BIC did not infect IT85F-2687 and Futo Coiled. SBMV and BCMV-BIC did not cause infection in IT86D-371, similarly, CMeV and SBMV did not cause infections CP-VAR8. CMeV and BCMV-BIC significantly ($p < 0.05$) reduced cowpea yield in 27 varieties, CABMV in 23 and SBMV in 14. Incidence of BCMV-BIC infection was 100% in 13 varieties, CABMV infection in 9, CMeV in 15 and SBMV in 3 varieties. IT82D-889, IT86D-880, CP-VAR8, IT85F-2687, IT86D-1010, IT90K-284-2 and Futo coiled were resistant or tolerant to the virus strains; these lines are potential breeding materials for cowpea viruses' resistance.

KEYWORDS: Cowpea, viruses, Disease incidence, Cowpea, *Vigna*, ELISA.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is an important legume crop in Africa and Asia. It contains 15 – 26% protein (Umoren, 1997), therefore it provides more than 60% the amount of dietary protein from plant sources for people in these areas (Quin, 1997). It is a valuable component of farming systems, for instance in Nigeria, small land-holders earn living through intercropping cowpea with maize, sorghum or millet, and use it as a cover crop to check erosion and weeds (Mortimore *et al.* 1997; Hutchinson and McGiffen, 2000). Estimate of world production of cowpea grain is 2.7 million tonnes per annum, which Nigeria, produces more than 60% (Quin, 1997).

Pathogens and pests such as viruses, bacteria, insects, nematodes and fungi cause serious yield losses in cowpea (Singh *et al.* 1990; Emechebe *et al.* 1991; Jackai and Adalla, 1997). Viruses infecting cowpeas are found all over the world and several studies have shown that virus infections reduce cowpea yield in the field by between 10 to 100% (Kaiser and Mossahebbi, 1975; Bozarth and Shoyinka, 1979; Taiwo and Shoyinka, 1988; Kannaiyan *et al.* 1993; Anderson *et al.* 1996). Shoyinka *et al.* (1997) reported that eight viruses infect cowpea in Nigeria and some of them are found in the major cowpea growing areas.

Although appropriate cultural practices such as management of vectors, weeding of alternative host plants, etc. can be employed to reduce the extent of yield losses by viral infection, but breeding of cowpea resistant varieties to viruses is the most sustainable approach to combat viral diseases. Planting resistant lines is cheaper for the farmers and environment friendly because pesticides, etc. are no longer used to control pests. The objective of this study was to identify cowpea lines resistant to some cowpea viruses as a basic step in breeding resistant varieties.

MATERIALS AND METHODS

Resources

Thirty-three cowpea lines (11 advanced cultivars, 16 breeding lines and 6 landraces) shown in Table 1 were screened in 1999 - 2000 for reactions to four cowpea viruses in an insect – free screen-house. The four viruses used in the study were Cowpea aphid-borne mosaic *potyvirus* (CABMV), Bean common mosaic *potyvirus*-blackeye cowpea strain (BCMV-BIC), Cowpea mottle *carmovirus* (CMeV) and Southern bean mosaic *sobemovirus* (SBMV).

Isolates of the viruses were obtained from the Virology Unit, International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria and were cultured by mechanically inoculating the viruses into disease free Ife brown variety grown in the screen house; until the viruses were needed.

Each cowpea variety was sown in ten Stewart's 8" plant pots (5 seeds per pot), the number of seedlings per pot were later thinned to two. Twenty seedlings of each variety were mechanically inoculated with isolates

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Tables 1. The status of cowpea lines used in the study and their source

S/N	Genotype	Status	Source
1.	Ife brown	Advanced cultivar	Virology Unit, IITA, Ibadan
2.	IT82D-716	Advanced cultivar	GRU, IITA, Ibadan
3.	IT82D-889	Advanced cultivar	GRU, IITA, Ibadan
4.	IT84S-2246-4	Advanced cultivar	GRU, IITA, Ibadan
5.	IT86D-371	Advanced cultivar	GRU, IITA, Ibadan
6.	IT86D-880	Advanced cultivar	GRU, IITA, Ibadan
7.	IT87D-784-1	Advanced cultivar	GRU, IITA, Ibadan
8.	IT96D-774	Advanced cultivar	Virology Unit, IITA, Ibadan
9.	IT97K-499-38	Advanced cultivar	Virology Unit, IITA, Ibadan
10.	TVu 12349	Advanced cultivar	Virology Unit, IITA, Ibadan
11.	TVx 3236	Advanced cultivar	Virology Unit, IITA, Ibadan
12.	ART.91-2	Breeding line	IAR and T, Ibadan
13.	CP-VAR8	Breeding line	CPEB, UI, Ibadan
14.	IAR 48	Breeding line	IAR and T, Ibadan
15.	IAR 72	Breeding line	IAR and T, Ibadan
16.	IT83D-442	Breeding line	Virology Unit, IITA, Ibadan
17.	IT85F-2687	Breeding line	Virology Unit, IITA, Ibadan
18.	IT85F-867-5	Breeding line	Virology Unit, IITA, Ibadan
19.	IT86D-1010	Breeding line	GRU, IITA, Ibadan
20.	IT86D-719	Breeding line	Virology Unit, IITA, Ibadan
21.	IT89KD-775	Breeding line	Virology Unit, IITA, Ibadan
22.	IT90K-284-2	Breeding line	GRU, IITA, Ibadan
23.	IT95K-1093-5	Breeding line	Virology Unit, IITA, Ibadan
24.	IT96D-740	Breeding line	Virology Unit, IITA, Ibadan
25.	IT97K-1068-7	Breeding line	Virology Unit, IITA, Ibadan
26.	IT97K-491-2	Breeding line	Virology Unit, IITA, Ibadan
27.	TVu 66	Breeding line	Virology Unit, IITA, Ibadan
28.	Futo coil	Landrace	CPEB, UI, Ibadan
29.	Solojo 2	Landrace	CPEB, UI, Ibadan
30.	Solojo 4	Landrace	CPEB, UI. Ibadan
31.	TVu 11426	Landrace	GRU, IITA, Ibadan
32.	TVu 1190	Landrace	GRU, IITA, Ibadan
33.	TVu 13686	Landrace	GRU, IITA, Ibadan

Key: GRU = Genetic Resources Unit; CPEB, UI = Department of Crop Protection and Environmental Biology, University of Ibadan; IAR and T = Institute of Agricultural Research and Training, Ibadan.

Table 2. Mean square values for the analysis of variance in 1999 and 2000 for disease symptoms and yield of cowpea lines infected with four viruses infecting cowpea.

Source of variation	DF	2WAI	6WAI	YIELD
Varieties	32	17.299^{**}	147.72^{**}	$3.5 \times 10^{6**}$
Viruses	4	169.1^{**}	710.86^{**}	$9.0 \times 10^{6**}$
Year	1	0.67	0.0426	1.1
Error	1485	0.247	0.538	13970.2
Total	1522			

Key: ** = means are significantly different at 0.01 level of significance.

of the viruses at the emergence of the first trifoliate leaf, control plants were not inoculated with any virus. The experiment was laid a 33 x 5 factorial experiment in a completely randomised designed; experimental pots were randomly placed in the screen house bench with random numbers. The four viruses and the control were the main effect and the varieties the minor effect.

Inoculation and screening

The cowpea lines were mechanically inoculated with inoculum of each virus by grinding young infected leaves from the stock culture plant (Ife brown) in ice cooled inoculation buffer. The buffer is made up of 1 g of dibasic potassium phosphate (K_2HPO_4), 1 g monobasic potassium phosphate (KH_2PO_4), 0.1 g of sodium sulphite (Na_2SO_3) in 100 ml distilled water at pH 7.5, (Walkey, 1985). Carborundum powder was sprinkled on the leaves and inoculum was gently rubbed in with gloved fingers. Excess inoculum was rinsed off with distilled water. The incidence of infection was calculated

based on the proportion of infected plants to the total number of the plants in 10 pots in the screen house. Disease symptoms were visually observed and recorded at the 2nd and 6th week after planting.

Serology

Plants which expressed no symptoms were tested for presence of viruses using Protein-A sandwich enzyme linked immunosorbent assay (PAS – ELISA) described by Hughes and Thomas (1988). Multi-well ELISA plates were coated with 100 µl Protein-A dissolved in a coating buffer (1.59 g sodium carbonate (Na_2CO_3), 2.93 g sodium bicarbonate ($NaHCO_3$) and 0.2 g of sodium nitrite (NaN_3) in 1 litre of distilled water at pH 9.6); then incubated at 37°C for 2 hours. The plates were washed thrice with phosphate buffered saline Tween (PBS Tween) containing 8 g of sodium chloride (NaCl), 0.2 g of monobasic potassium phosphate (KH_2PO_4), 1.1 g of sodium phosphate (Na_2HPO_4), 0.2 g of potassium chloride (KCl); 0.2 g sodium nitrite (NaN_3) in 5 ml of distilled water at pH 7.4. 100 µl of appropriate polyclonal antibody dissolved in 5 ml of PBS Tween was trapped onto the plates and incubated at 37°C for 2 hours, then washed thrice in PBS Tween. Extracts of virus infected leaf samples were squashed in PBS Tween and 2% PVP (polyvinyl pyrolidone) and 100 µl squashed extracts were pipetted into wells of the ELISA-plates and incubated at 4°C overnight. The plates were washed three times with PBS Tween in the morning of the second day. 100 µl of polyclonal antibody was added again to sandwich the virus (antigen). 100 µl of Protein-A alkaline phosphate diluted in conjugate buffer was added to each well. Conjugate buffer contains 50% PBS Tween, 0.02% egg albumin, 0.02% PVP and 0.02% NaN_3. The plates were incubated at 37°C for 2 hours, and then washed three times in PBS Tween. 200 µl of p-nitrophenyl phosphate (pNPP) was dissolved in substrate buffer at 1 µg/ml containing 97 ml diethanolamine, 0.2 g NaN_3, 800 ml of distilled water at pH 9.8. The plates were read twice with DYNEX MRX microplate reader, the first reading was taken after one hour while the second was read after the plates stood overnight on the laboratory bench.

Polyclonal antisera to the viruses were obtained from the Virology Unit, IITA, Ibadan Nigeria. Disease severity was scored visually using a 5 – point scale proposed by Thottappilly *et al.* (1994)

1 = no infection (no symptom was observed on the

Table 3. Responses of cowpea lines to infection by four cowpea viruses in 1999 and 2000.

S/N	VARIETIES	BCMV-BIC	CABMV	CMeV	SBMV
1	Ife brown	SS,SD,N	SS,SD,	SS	SS,CH
2	IT82D-716	SS	SS,CH	SS,SD	LL
3	IT82D-889	-	-	SS,CH	-
4	IT84S-2246-4	SS,CH	SS,CH	SS,SD	SS
5	IT86D-317	-	SS	SS	-
6	IT86D-880	-	-	SS,CH	LL
7	IT87D-784-1	SS	SS,CH	SS,CH	SS
8	IT96D-774	SS,CH	SS	SS,SD	SS
9	IT97K-499-38	SS,SD,N	SS,SD	SS	SS
10	TVu 12349	SS	SS,CH	SS	SS
11	TVX 3236	SS,SD,CH,N	SS,SD,CH	SS,SD	SS
12	ART 91-2	SS,SD	SS,SD	SS,N	SS
13	CP-VAR8	SS	SS	-	-
14	IAR 48	SS,N	SS,CH	SS,N	SS,CH
15	IAR 72	SS,N	SS,CH	SS,N	SS,CH
16	IT83D-442	SS,SD	SS,SD	SS,SD	SS
17	IT85F-2687	-	-	SS	LI
18	IT85F-867-5	SS,N	SS,SD,CH	SS	SS
19	IT86D-1010	LI	-	LI	SS
20	IT86D-719	SS,CH	SS	SS,SD	LL
21	IT89KD-775	SS,SD	SS,SD	SS	SS
22	IT90K-284-2	-	-	SS,SD	-
23	IT95K-1093-5	SS,SD	SS	SS	-
24	IT96D-740	SS,SD	SS,CH	SS	SS
25	IT97K-1068-7	SS,SD	SS,CH	SS	-
26	IT97K-491-2	SS,SD	SS,SD	SS,CH	SS,CH
27	TVu 66	SS,SD	SS	SS,SD	SS
28	Futo Coiled	-	-	LI	LL
29	Solojo 2	SS,CH,N	SS	LI	LL
30	Solojo 4	SS,SD	SS	SS	SS
31	TVu 11426	SS	LI	SS,SD	LI
32	TVu 1190	SS,SD	LI	SS	SS
33	TVu 13686	SS,SD	-	SS,SD	SS

CH =Chlorosis; LI = Latent Infection; LL = Local Lesion; SS = Systemic Symptoms; N = Necrosis; SD = Stunting/Dwarfing; - = No infection.

Tables 4. Infection severity and disease incidence of four seed-transmitted viruses on some cowpea varieties in the screen house in 1999 and 2000.

S/N	VARIETY	BCMV-BIC		CABMV		CMeV		SBMV	
		Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	incidence
1	Ife brown	5	100.0	5	100.0	3	100.0	5	86.7
2	IT82D-716	5	100.0	3	57.1	5	100.0	5	87.5
3	IT82D-889	1	0.0	1	0.0	3	60.0	1	0.0
4	IT84S-2246-4	5	100.0	5	100.0	5	100.0	4	92.9
5	IT86D-317	1	0.0	2	12.5	4	77.8	1	0.0
6	IT86D-880	1	0.0	1	0.0	5	100.0	3	58.3
7	IT87D-784-1	3	58.3	4	63.6	5	100.0	4	92.9
8	IT96D-774	5	93.3	4	100.0	5	100.0	3	66.7
9	IT97K-499-38	5	100.0	5	100.0	4	100.0	3	37.5
10	TVu 12349	5	61.5	4	92.3	2	33.3	2	43.8
11	TVX 3236	5	100.0	4	100.0	4	66.7	3	77.8
12	ART 91-2	3	83.3	5	100.0	3	54.5	2	27.3
13	CP-VAR8	5	71.4	5	73.3	1	0.0	1	0.0
14	IAR 48	4	100.0	5	77.8	3	60.0	2	50.0
15	IAR 72	5	100.0	2	87.5	5	100.0	4	100.0
16	IT83D-442	4	100.0	3	85.7	2	66.7	2	14.3
17	IT85F-2687	1	0.0	1	0.0	2	28.6	2	20.0
18	IT85F-867-5	5	88.9	2	77.8	2	50.0	3	28.5
19	IT86D-1010	2	16.6	1	0.0	5	100.0	2	40.0
20	IT86D-719	4	100.0	4	63.6	3	83.3	4	91.7
21	IT89KD-775	3	55.5	3	85.7	3	88.9	3	60.0
22	IT90K-284-2	1	0.0	1	0.0	4	100.0	1	0.0
23	IT95K-1093-5	5	90.9	4	91.7	4	100.0	1	0.0
24	IT96D-740	5	90.0	5	88.9	5	100.0	2	23.5
25	IT97K-1068-7	5	100.0	3	100.0	4	100.0	1	0.0
26	IT97K-491-2	5	100.0	5	100.0	5	100.0	3	33.3
27	TVu 66	5	100.0	5	100.0	3	64.3	4	83
28	Futo Coiled	1	0.0	1	0.0	4	81.8	3	40.0
29	Solojo 2	5	61.5	5	64.3	2	33.3	5	100.0
30	Solojo 4	4	54.5	4	85.7	2	14.3	3	80.0
31	TVu 11426	3	63.6	2	66.7	3	25.0	2	20.0
32	TVu 1190	5	100.0	2	7.1	2	83.3	2	9.1
33	TVu 13686	4	90.9	1	0.0	5	100.0	4	100.0

Key: 1 = no infection; 2 = light infection; 3 = moderate infection 4 = severe infection; 5 = very severe infection

leaves and ELISA result was negative),
2 = light infection (0 – 20% of the leaves expressed symptoms and ELISA result of symptomless plants was positive),
3 = moderate infection (symptoms appeared on 21 – 40% of the leaves),
4 = severe infections (symptoms appears on 41-60% of the leaves),
5 = very severe infections (symptoms appeared on more than 60% of the leaves).

Statistcal analysis

An analysis of variance of data for the 2 years was computed. The disease severity score was transformed with $\log_{10}$ of disease score + 1. Comparisons of means were done using least square means (LSMeans) and associated standard errors, using pairwise differences (pdiff) of means option in statistical analysis system (SAS) (SAS institute, 1995).

RESULTS AND DISCUSSION

The mean square values from the analysis of variance are shown in Table 2. There was no significant ($p < 0.05$) difference in symptoms expression at the second and sixth week after inoculation of the viruses and in yield in 1999 and 2000. The viruses caused systemic symptoms in most of the diseased plants (Table 3). The common symptoms induced by all the viruses were mosaic patterns, mottling, poor pod formation, foliate distortion and defoliation. Similar symptoms were reported by Bock (1973), Shoyinka (1974) Thottappilly and Rossel (1992). BCMV-BIC caused stunting or dwarfing in 13 varieties, CABMV in 7, CMeV in 10 but SBMV did not cause stunting in any variety. BCMV-BIC caused premature death of some plants in Ife brown, IT97K-499-38, TVx 3236, IAR 48, IAR 72, IT85F-867-5 and Solojo 2 and CMeV in ART 91-

Table 5. Comparison of seed yield (Kg/Ha) of cowpea varieties infected with four cowpea viruses.

S/N	VARIETY	BCMV-BIC	CABMV	CMeV	SBMV	CONTROL
1	Ife brown	145.84^{c}	211.60^{c}	184.30^{c}	631.99^{b}	1152.41^{a}
2	IT82D-716	140.04^{b}	165.33^{b}	154.06^{b}	188.23^{b}	882.45^{a}
3	IT82D-889	89.70^{b}	679.06^{a}	99.03^{b}	734.90^{a}	732.49^{a}
4	IT84S-2246-4	136.52^{b}	166.54^{b}	188.00^{b}	205.66^{b}	693.82^{a}
5	IT86D-317	141.33^{c}	108.19^{c}	132.58^{c}	395.85^{b}	864.43^{a}
6	IT86D-880	1007.10^{a}	893.16^{a}	69.09^{b}	1006.27^{a}	955.03^{a}
7	IT87D-784-1	522.60^{b}	611.07^{b}	589.66^{b}	705.65^{b}	1008.12^{a}
8	IT96D-774	89.60^{c}	152.55^{b}	77.52^{c}	169.36^{b}	938.58^{a}
9	IT97K-499-38	189.36^{c}	223.12^{c}	150.44^{c}	566.41^{b}	1021.44^{a}
10	TVu 12349	196.54^{c}	611.55^{b}	177.35^{c}	720.42^{ab}	821.41^{a}
11	TVX 3236	78.54^{c}	562.00^{b}	198.55^{c}	842.24^{a}	862.01^{a}
12	ART 91-2	500.14^{b}	323.14^{c}	402.25^{bc}	845.04^{a}	862.36^{a}
13	CP-VAR8	253.02^{b}	362.11^{b}	877.26^{a}	912.12^{a}	856.96^{a}
14	IAR 48	322.42^{b}	401.23^{b}	356.44^{b}	785.47^{a}	866.47^{a}
15	IAR 72	241.36^{c}	658.11^{b}	189.00^{c}	599.68^{b}	954.58^{a}
16	IT83D-442	234.67^{c}	344.71^{c}	98.52^{d}	874.54^{b}	1520.44^{a}
17	IT85F-2687	804.55^{a}	896.47^{a}	787.58^{a}	869.55^{a}	794.23^{a}
18	IT85F-867-5	611.78^{b}	988.84^{a}	788.26^{a}	820.20^{a}	894.01^{a}
19	IT86D-1010	1038.46^{a}	1252.47^{a}	296.45^{b}	1084.22^{a}	1189.62^{a}
20	IT86D-719	446.24^{b}	526.20^{b}	502.22^{b}	479.26^{b}	852.72^{a}
21	IT89KD-775	231.65^{b}	342.13^{b}	186.66^{b}	320.40^{b}	936.24^{a}
22	IT90K-284-2	865.42^{a}	823.04^{a}	886.23^{a}	844.46^{a}	868.66^{a}
23	IT95K-1093-5	322.46^{c}	645.31^{b}	298.24^{c}	922.02^{a}	887.60^{a}
24	IT96D-740	466.28^{b}	522.43^{b}	486.12^{b}	765.81^{a}	824.55^{a}
25	IT97K-1068-7	96.32^{c}	422.85^{b}	426.12^{b}	786.36^{a}	842.23^{a}
26	IT97K-491-2	112.30^{c}	482.26^{b}	98.44^{c}	520.43^{b}	828.91^{a}
27	TVu 66	76.85^{c}	322.41^{b}	88.56^{c}	861.42^{a}	903.12^{a}
28	Futo Coiled	1044.22^{a}	982.64^{a}	423.45^{b}	965.63^{a}	1224.51^{a}
29	Solojo 2	392.65^{c}	503.66^{bc}	664.55^{b}	688.14^{b}	924.45^{a}
30	Solojo 4	296.25^{c}	633.57^{b}	344.52^{c}	800.56^{b}	1423.28^{a}
31	TVu 11426	795.25^{a}	842.23^{a}	805.50^{a}	832.33^{a}	911.53^{a}
32	TVu 1190	521.63^{b}	867.41^{a}	856.00^{a}	988.62^{a}	865.75^{a}
33	TVu 13686	84.66^{b}	676.85^{a}	140.66^{b}	720.92^{a}	684.22^{a}

Key: Means with the same letter superscript across the rows are not significantly different at 5% probability (L SMeans).

2, IAR 48 and IAR 72, while CABMV and SBMV did not cause the death of any plant in any of the varieties. Latent infection was observed in IT86D-1010, Futo coiled and Solojo 2 infected with CMeV, also in IT86D-1010 infected with BCMV-BIC, TVu 11426 and TVu 1190 infected with CABMV, and IT85F-2687 and TVu 11426 infected with SBMV, these cowpea varieties are therefore tolerant to the respective cowpea viruses.

None of the thirty-three cowpea varieties escaped infection from the four viruses. Whereas IT86D-719, IT96D-740, Ife brown, IT82D-716 and IT84S-2246-4 were very severely infected by all the four viruses, IT90k-284-2 and IT82D-889 were not infected by BCMV-BIC, CABMV and SBMV (Table 4). BCMV-BIC did not cause infection in 6 varieties (IT82D-889, IT86D-317, IT86D-880, IT85F-2687, IT90K-284-2 and Futo coiled), CABMV did not cause infections in 7 varieties, CMeV in one (CP-VAR 8) and SBMV in 6. Incidence of virus infection ranged from 0 to 100% in the various lines.

Table 5 compares seed yield of cowpea varieties following infection with the viruses. BCMV-BIC significantly ($p < 0.05$) reduced the yield in 27 varieties, CABMV in 23, CMeV in 27 and SBMV in 14 varieties. CMeV and BCMV-BIC significantly ($p < 0.05$) reduced the yield in severely and very severely infected varieties than CABMV and SBMV.

In conclusion, IT90K -284-2 and IT82D-889 were distinguished as potential breeding materials for resistance to three viruses (BCMV-BIC, CABMV and SBMV).

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