Asian Journal of Plant Pathology

ISSN 1819-1541

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Plant Pathology 9 (2): 83-90, 2015
ISSN 1819-1541 / DOI: 10.3923/ajppaj.2015.83.90

Serological Screening of Cowpea Genotypes for Resistance against Cowpea Aphid Borne Mosaic Virus Using DAS-ELISA

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ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp), an important protein rich arid legume crop is susceptible to number of fungal, bacterial and viral diseases that severely limit the productivity. A set of 92 diverse cowpea genotypes including varieties, mutants, advanced breeding lines, exotic and indigenous collections were serologically screened for resistance against Cowpea Aphid Borne Mosaic (CABM) virus using DAS-ELISA. The genotypes grown in triplicate in a randomized block design at Trombay were mechanically inoculated with CABM virus and the resistance or susceptible reactions of each of the genotypes were recorded visually as well as serologically. Based on the extent of symptoms and serological reactions, the cowpea genotypes were classified as: highly resistant, plants without symptoms and negative for serology; resistant, plants with mild mosaic (<25%) and positive for serology; susceptible, plants with mosaic (26-75%) and positive for serology and highly susceptible, plants with severe mosaic (>75%) and other systemic symptoms and positive for serology. The study resulted in the identification of 13 highly resistant, 24 resistant, 50 susceptible and 5 highly susceptible genotypes. The chlorophyll index of susceptible genotypes as measured by SPAD chlorophyll meter was almost half that of resistant genotypes. The highly resistant genotypes against CABM virus identified in the present study after due confirmation would be incorporated in the breeding programme to develop resistance in elite genetic backgrounds.

Key words: Cowpea, ELISA, CABMV, mechanical transmission, serology

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is an important arid legume crop widely cultivated in the arid and semi-arid regions of the world and known for its high-quality dietary protein, acceptable palatability and low cost of production. Even though, cowpea is bestowed with tolerance to abiotic factors like drought, they are susceptible to biotic stresses like diseases that impede the expression of its tangible genetic yield potential.

Among the diseases infecting cowpea, those caused by viruses are devastating and are known to bring about yield losses ranging from 10-100% (Rachie, 1985). Though over 140 viruses have been identified as naturally infecting cowpea (Shoyinka *et al*., 1997; Hughes and Shoyinka, 2003), about 20 viruses possessing RNA genomes are of major occurrence worldwide (Hampton *et al*., 1997).

Cowpea Aphid-Borne Mosaic Virus (CABMV), a ssRNA virus belonging to the genus *Potyvirus*, is one of the economically significant and cosmopolitan viruses known to inflict severe yield losses in cowpea. This seed-borne distinctive virus with flexuous filamentous particles, 750×12 nm (Damiri *et al*., 2013), is transmitted in a stylet-borne, non-persistent manner by several common species of aphids such as *Aphis craccivora*, *A. fabae, A. gossypii*, *A. medicaginis*, *Macrosiphum euphorbiae* and *Myzus persicae*. CABMV has a wide experimental host range and known to infect many species in the Leguminosae, Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Labiatae and Solanaceae families (Bock, 1973; Damiri *et al*., 2013). The CABMV with wide geographical distribution has been reported from almost all the continents, where, cowpea is grown (Damiri *et al*., 2013).

The most economical, practicable and effective method of control of legume viruses is through the use of resistant varieties (Taiwo, 2003). Development of resistant varieties against different type and strain of viruses entails screening of germplasm in a particular agro-climate for identification of resistance to the particular strain prevailing in that region.

Among the screening methods, enzyme-linked immunoassays have become the principal ones, being highly sensitive, relatively simple to use and suited for large scale testing (Albrechsten, 2006). The DAS-ELISA based serological survey to identify the major virus prevailing at Trombay was carried out earlier as a prelude to disease resistance breeding. The CABMV was found to be the predominant virus followed by Cucumber Mosaic Virus (CMV) and cowpea mosaic viruses. The CMV was also found to co-exist along with CABMV in most of the infections (Table 1).

Disease scoring primarily based on the symptoms and the lack of assurance that all the genotypes have been exposed to viral inoculums are considered as major limitations in screening of virus resistance under field conditions. Therefore, in a manner to contravene these logjams, an attempt was made to screen 92 diverse cowpea genotypes for resistance against CABMV under field conditions by resorting to mechanical inoculation and serological testing for presence or absence of the virus post inoculation.

MATERIALS AND METHODS

Plant material: Ninety-two cowpea genotypes comprising of germplasm lines, mutants, advanced breeding lines and varieties were sourced from different cowpea growing regions for use in the present study and are listed in Table 2.

Table 1: Identification of viruses infecting cowpea genotypes under field condition using DAS-ELISA kits/RT-PCR

Virus kit	Genotypes showing positive serology	Symptom range
CABMV	ARC-1, BLRC1, BLRC4, BLRC5, BLRC8, BLRC10, BLRC11, BLRC13,BLRC15, BLRC16, BLRC17, BLRC18, BLRC23, Cowpea Local, CPD103, CPD118, DCS47-1, EC394736, EC394763, EC536635, GC3, GC4, GC521, IC202784, IC366776, IC402172, JOB129, KBC2, PCGP1, PGCP3, PGCP5, PGCP6, PGCP11, Sarika, TVX994-1	Vein banding, vein clearing, mosaic, leaf curl (downward and upward), interveinal chlorosis, slight crinkling, chlorotic patches on flowers and pods and mottling
CPMV	BLRC2, BLRC8, BLRC13, C152, CPD118, IC202784, IC366776, KM5	Mosaic, interveinal chlorosis and vein banding
CPSMV	ARC1, PGCP3	Crinkling, curling, severe mosaic and chlorosis
CPMMV	CPD103	Mottling, chlorosis, stunting and reduced leaf size
CCMV	BLRC2	Vein banding and interveinal chlorosis
CPMoV	None	
CMV (RT-PCR)	ARC-1, BLRC1, BLRC5, BLRC8, BLRC17, BLRC23, Cowpea Local, EC536635, GC3, GC4, GC521, IC202784, IC366776, IC402172, JOB129, KBC2, PGCP6, Sarika, TVX994-1, TC601, TC901, V585	Mottling, vein banding and vein clearing, interveinal chlorosis and mosaic

CABMV: Cowpea aphid borne mosaic virus, CPMV: Cowpea mosaic virus, CPSMV: Cowpea severe mosaic virus, CPMMV: Cowpea mild mottle virus, CCMV: Cowpea chlorotic mottle virus, CPMoV: Cowpea mottle virus, CMV: Cucumber mosaic virus

Table 2: List of cowpea genotypes collected from different locations

State	No. of genotypes	Genotypes
Kerala	5	Anaswara, Baghya Lakshmi, Kanakamony Lola, Sarika
Karnataka	28	Arka Suman, BLRC1, BLRC2, BLRC3, BLRC4, BLRC5, BLRC6, BLRC7, BLRC8, BLRC9, BLRC10, BLRC11, BLRC12, BLRC13, BLRC14, BLRC15, BLRC16, BLRC17, BLRC18, BLRC19, BLRC20, BLRC21, BLRC22, BLRC23, C152, KBC2, KM5, TVX994-1
Tamil Nadu	4	CO2, CO4, CO6, COCP7
Gujarat	7	DCS47-1, GC3, GC4, GC5, GC502, GC510, GC521
Rajasthan	6	CPD103, CPD115, CPD118, CPD91, JOB129, RC101
Maharashtra	16	ARC-1, Cowpea Local, TC1-26-E, TC1-6-10-1, TC1-6-10-2, TC1-6-9-E, TC201, TC501-1-1, TC501-1-4, TC503(L), TC601, TC605, TC901, TC99-1, TC99-9, TCM418SDT
Delhi	3	V585, NBC1, NBC3
Exotic	4	EC394736, EC394763, EC517140, EC536635
Indigenous	6	IC202784, IC202797, IC366776, IC402172, IC402175, IC521495
IITA, Nigeria	4	IT00K-1197, IT38956-1, IT86F-2014-1, IT86F-20895-1
Uttarakhand	8	PGCP1, PGCP3, PGCP5, PGCP6, PGCP11, PGCP12, PGCP13, PGCP14
Goa	1	Alsando

Virus maintenance and preparation of sap inoculum: The virus was obtained in March, 2013 from symptomatic cowpea plants from experimental field at Trombay. Virus isolates were propagated and maintained in cowpea plants in isolation in growth chambers maintained at 25-27°C. Sap was extracted by triturating symptomatic leaves with a mortar and pestle in cold 0.1 M Tris-buffer, pH 7.0 containing 0.0005 M EDTA trisodium salt as stabilizing additive.

Screening for CABMV resistance by sap inoculation: The 92 genotypes were screened in field by sap inoculation method to confirm their resistance against CABMV. Five plants per replication per genotype were grown in a completely randomized block design in triplicate. The primary leaves of test plants were inoculated by a conventional leaf rub method with a cotton swab and carborundum (800 mesh) as an abrasive. Inoculations were repeated twice and the plants were observed for a month. Observations related to presence or absence of symptoms, nature and extent of symptoms were recorded. Data on chlorophyll index were also recorded on ten resistant and ten susceptible genotypes using SPAD 502 chlorophyll meter (Konica Minolta) and was used to compare the reduction in chlorophyll content of the susceptible lines against that of the resistant lines.

Serological screening by DAS-ELISA: The presence or absence of the virus in the inoculated plants was confirmed serologically by using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977), with the help of kits obtained from AC Diagnostics Inc. (USA). Three expanding leaves from each of five plants (symptomatic plants wherever possible) per genotype were collected and were crushed in mortar and pestle using extraction buffer provided in the kit. The crushed extract was filtered using muslin cloth and the filtrate was stored in 12 mL polypropylene tubes at -20°C for use in serological testing.

Serological testing followed the manufacturer's protocol. The controls consisted of a blank (extraction buffer without plant sap), negative control (healthy cowpea leaf samples) and positive control (leaf samples from infected cowpea plant). The last two were supplied together with the ELISA kits. After addition of p-nitrophenyl phosphate substrate (1 mg mL^{-1} in 10% diethanolamine, pH 9.8) and incubation for 30 min at room temperature in the dark, ELISA reactions (absorbencies) were measured using an universal automated microplate reader ELX800Ms (Bio-Tek Instruments Inc., USA) at 405 nm. A sample was considered virus infected if its A_{405} nm value was at least twice that of negative control (Damiri *et al*., 2013).

Classification of resistance: According to the symptoms and the serological results, the cowpea genotypes inoculated with the virus isolates were classified as: Highly resistant; plants without symptoms and negative for serology, resistant; plants with mild mosaic (<25%) and positive for serology, susceptible, plants with mosaic (26-75%) and positive for serology and highly susceptible; plants with severe mosaic (>75%) and other systemic symptoms and positive for serology.

RESULTS

Screening for CABMV resistance by sap inoculation: The leaf extract or sap isolated from the CABMV infected plants maintained in isolation in growth chamber was used for inoculation of the primary leaves of each plant. Inoculation was done with 2 mL of the sap twice at 2 days interval. The inoculated plants articulated the symptoms 12-17 days post sap inoculation. The symptoms were expressed on the 2nd or 3rd subsequent leaf from the inoculated leaf (Fig. 1). The manifestation of infection showed a wide range of symptoms across the genotypes that included vein banding, vein clearing, mosaic, upward or downward leaf curl, interveinal chlorosis, crinkling, mottling and chlorotic patches on flowers and pods. The disease severity ranged from 0-100% (Table 3).

Table 3: Screening of cowpea genotypes for resistance against CABMV under field conditions by sap inoculation and confirmation by DAS-ELISA

Disease severity (%)	Genotypes	ELISA reaction
0 (Highly resistant)	CO6, IC521495, IT86F-2014-1, IT86F-20895-1, PGCP12, RC101, TC1-6-10-1, TC1-6-9-E, TC501-1-4, TC503, TC605, TC99-1, TCM418SDT	-
1-25 (Resistant)	Anaswara, ArkaSuman, Bhagya Lakshmi, BLRC9, BLRC11, BLRC22, COCP7, EC517140, IC402172, IC402175, IT38956-1, Kanakamony, Lola, PGCP13, PGCP14, PGCP6, Sarika, TC1-26-E, TC1-6-10-2, TC201, TC501-1-1, TC601, TC99-9, TC901	+
26-75 (Susceptible)	Alsando, ARC1, BLRC1, BLRC10, BLRC12, BLRC13, BLRC14, BLRC15, BLRC16, BLRC17, BLRC18, BLRC19, BLRC2, BLRC20, BLRC21, BLRC23, BLRC3, BLRC4, BLRC5, BLRC6, BLRC7, BLRC8, C-152, CO2, CO4, CPD115, CPD118, CPD91, DCS47-1, EC394763, EC394736, EC536635, GC3, GC4, GC502, GC521, IC202784, IC202797, IT00K-1197, JOB129, KBC2, KM5, NBC1, NBC3, PGCP1, PGCP11, PGCP3, PGCP5, TVX944-1,V585	+
76-100 (Highly susceptible)	C.Local, CPD103, GC5, GC510, IC366776	+

Fig. 1: Sap transmission of CABM virus

CPD103 (Highly susceptible) TC1-6-10-1 (Highly resistant)

Fig. 2: Highly susceptible and highly resistant genotypes for CABM virus disease following sap inoculation

Table 4: Mean chlorophyll index (SPAD) of highly resistant and highly susceptible cowpea genotypes for CABMV

Immune/resistant genotype	Mean SPAD reading	Highly susceptible/susceptible genotype	Mean SPAD reading
CO6	53.54	C. Local	27.00
IC521495	49.48	CPD103	11.78
IT86F-2014-1	54.70	GC510	22.18
IT86F-20895-1	51.52	IC366776	43.94
RC101	57.46	GC4	22.06
TC1-6-10-1	52.34	GC5	29.80
TC501-1-4	57.56	GC521	32.00
TC503	60.30	JOB129	26.74
TC605	56.88	CPD118	26.88
TC99-1	58.50	V585	30.68
Mean±SE	55.23±1.10	Mean	27.31±2.61

Only the symptomatic plants showed the presence of the virus serologically. The presence or absence of CABM virus in the sap inoculated plants was also confirmed serologically. Based on the symptoms and the serological results for CABMV screening using sap inoculation, 13 genotypes (CO6, IC521495, IT86F-2014-1, IT86F-20895-1, PGCP12, RC101, TC1-6-10-1, TC1-6-9-E, TC501-1-4, TC503, TC605, TC99-1, TCM418SDT), were classified as highly resistant, 24 resistant, 50 susceptible and 5 highly susceptible (Table 3 and Fig. 2).

The assessment of the degree of chlorophyll disintegration in susceptible genotypes owing to CABMV infection was carried out and the SPAD values of resistant genotypes was found to range from 49.48-60.30 with a mean value of 55.23, while in susceptible genotypes it was in the range of 11.78-43.94 with a mean of 27.31 (Table 4). Thus, the mean SPAD values of susceptible genotypes was almost half that of resistant genotypes; suggesting 50% disintegration of chlorophyll as a result of infection.

DISCUSSION

The success of disease resistance breeding relies on precise identification of pathogen and accurate screening of germplasm lines for a particular disease causing pathogen. The CABMV

belonging to genus *Potyvirus*, has been accounted as the most widespread and important constraint on cowpea crop in all of cowpea grown agro-ecological zones (Emechebe and Lagoke, 2000; Bashir *et al*., 2002). Consequently, the present investigation aimed at identifying putative resistance sources against CABM virus, the most prevalent virus at Trombay. The wide range of viral symptoms reported herein including vein banding, vein clearing, interveinal chlorosis, mosaic, yellowing, mottling, curling and crinkling are in conformity with earlier reports in cowpea (Salem *et al*., 2010). The key symptom of dark green vein-banding induced by CABMV in this study is also in accordance to that reported by Bock and Conti (1974). The sap transmissible nature of the virus (Brunt *et al*., 1990) was also confirmed in our study. But, field screening for virus resistance cannot be construed solely based on symptoms (Shoyinka *et al*., 1997), as different viruses display overlapping symptoms perceivable from the present study. Moreover, plants can also exhibit virus-like symptoms in retortion to adverse weather conditions, soil nutrient imbalances, non-viral infection and pest infestation (Naidu and Hughes, 2003). In addition, the exposure of the genotypes to viral inoculums under field conditions is also questionable. Therefore, to ensure accurate field diagnosis of virus infection, it is imperative to do confirmatory tests in conjunction with scrutiny of symptoms.

Particle morphology or serology (Bock and Conti, 1974) is indispensable for unequivocal identification of viruses. Consequently, DAS-ELISA based serological kit was employed for screening and identification of CABMV resistant genotypes. It was found that only symptomatic plants showed presence of virus serologically. The CABMV has been reported in many countries in different continents including Asia, Africa, Europe, North and South America and Australia (Mali and Kulthe, 1980; Huguenot *et al*., 1993; Bashir and Hampton, 1996; Pio-Ribeiro *et al*., 2000; Behncken and Maleevsky, 1977). Since, biological properties of CABMV may differ among isolates worldwide (Bashir *et al*., 2002); it becomes imperative to identify resistance sources against the local prevalent viral strains. Therefore, screening of genotypes using sap inoculation of the prevalent CABMV strain was carried out and has resulted in the identification of 13 highly resistant genotypes against this virus (Table 3). The previous reports on identification of resistance sources against CABMV in Central India are quite old (Mali *et al*., 1981) and the persistence of resistance in these genotypes considering the frequent mutational evolution rates in viruses, is questionable. Moreover, the resistant sources identified in the present study have not been reported earlier and could be used for introgression of resistance against the prevailing strains.

Under field conditions, mixed infections with more than one virus have been observed in cowpea (Pio-Ribeiro *et al*., 1978; Lima *et al*., 2005). As a result, selection of cowpea cultivars with multiple resistances is fundamental to control mixed infections (Anderson *et al*., 1996). Alternatively, it has been reported that under mixed infections, when symptoms are severe, one of the infecting virus generally is a *Potyvirus* (Kareem and Taiwo, 2007). Therefore, breeders should use cowpea genotypes with high resistance to viruses from the genus *Potyvirus* in the production of cowpea cultivars resistant to mixed infection (Lima *et al*., 2011). The CABMV resistant genotypes proclaimed in the present study could therefore be utilized to develop resistance against multiple viral infections. It is also accounted that early infection of virus severely impedes the crop productivity (Kareem and Taiwo, 2007). The near to 50% reduction in chlorophyll content of infected leaves vis-à-vis healthy plants in the present study could also attribute to severe reduction in yield of infected plants owing to reduced photosynthetic efficiency.

The use of host plant resistance against a particular disease depends extensively on accurate phenotyping and identification of proper resistance source. The putative resistance sources against CABMV, identified in the present investigation after due confirmation using molecular techniques

(RT-PCR) could be suitably incorporated in to disease resistance breeding for genetic improvement of cowpea. Furthermore, the resistant genotypes could be used to develop mapping population for tagging and development of molecular markers for use in marker assisted selection.

ACKNOWLEDGMENTS

The authors are thankful to Project Coordinator, National Network Project on Arid Legumes, ICAR; University of Agricultural Sciences, Bangalore; Sardar krushi nagar Dantiwada Agricultural University, Gujarat; Tamil Nadu Agricultural University, Coimbatore and National Bureau of Plant Genetic Resources, New Delhi for supply of cowpea genotypes. The authors also express their appreciation to Mr. P.N. Thokal, NABTD, BARC, for his technical assistance.

REFERENCES

Albrechsten, S.E., 2006. Testing Methods for Seed-transmitted Viruses: Principles and Protocols. CABL Publishing, Oxford, UK., ISBN: 9781845931483, Pages: 268.

Anderson, E.J., A.S. Kline, T.E. Morelock and R.W. McNew, 1996. Tolerance to Blackeye cowpea mosaic potyvirus not correlated with decreased virus accumulation or protection from cowpea stunt disease. Plant Dis., 80: 847-852.

Bashir, M. and R.O. Hampton, 1996. Detection and identification of seed-borne viruses from cowpea (*Vigna unguiculata* (L.) Walp.) germplasm. Plant Pathol., 45: 54-58.

Bashir, M., Z. Ahmad and A. Ghafoor, 2002. Cowpea aphid-borne mosaic potyvirus: A review. Int. J. Pest Manage., 48: 155-168.

Behncken, G.M. and L. Maleevsky, 1977. Detection of cowpea aphid-borne mosaic virus in Queensland. Anim. Prod. Sci., 17: 674-678.

Bock, K.R., 1973. East African strains of cowpea Aphid-borne mosaic virus. Ann. Applied Biol., 74: 75-83.

Bock, K.R. and M. Conti, 1974. Cowpea aphid borne mosaic virus. AAB Descriptions of Plant Viruses 134, July 1974. http://www.dpvweb.net/dpv/showdpv.php?dpvno=134.

Brunt, A., K. Crabtree and A. Gibbs, 1990. Viruses of Tropical Plants. CAB International, Wallingford, UK.

Clark, M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. General Virol., 34: 475-483.

Damiri, B.V., I.M. Al-Shahwan, M.A. Al-Saleh, O.A. Abdalla and M.A. Amer, 2013. Identification and characterization of Cowpea aphid-borne mosaic virus isolates in Saudi Arabia. Plant Pathol., 95: 79-85.

Emechebe, A.M. and S.T.O. Lagoke, 2000. Recent Advances in Research on Cowpea Diseases. In: Challenges and Opportunities for Enhancing Sustainable Cowpea Production, Fatokun, C.A., S.A. Tarawali, B.B. Singh, P.M. Kormawa and M. Tamo (Eds.). IITA, Ibadan, pp: 94-123.

Hampton, R.O., G. Thottappilly and H.W. Rossel, 1997. Viral Disease of Cowpea and their Control by Resistance-Conferring Genes. In: Advances in Cowpea Research, Singh, B.B., D.R.M. Raj, K.E. Dashiell and L.E.N. Jackal (Eds.). International Institute of Tropical Agriculture, Ibadan, pp: 159-175.

Hughes, J.A. and S.A. Shoyinka, 2003. Overview of Viruses of Legumes other than Groundnut in Africa. In: Plant Virology in Sub-Sharan Africa, Hughes, J.A. and J. Odu (Eds.). International Institute of Tropical Agriculture, Ibadan, Nigeria, pp: 553-568.

Huguenot, C., M.T. Furneaux, G. Thottappilly, H.W. Rossel and R.I. Hamilton, 1993. Evidence that cowpea aphid-borne mosaic and blackeye cowpea mosaic viruses are two different potyviruses. J. Gen. Virol., 74: 335-340.

Kareem, K.T. and M.A. Taiwo, 2007. Interactions of viruses in Cowpea: Effects on growth and yield parameters. Virol. J., Vol. 4, 10.1186/1743-422X-4-15

Lima, J.A.A., I.M. Sittolin and R.C.A. Lima, 2005. Diagnose e Estrategias de Controle de Doencasocasionadaspor Virus. In: Feijao-caupi: Avancos Tecnologicos, Filho, F.R.F., J.A. de Araujo Lima and V.Q. Ribeiro (Eds.). EmbrapaInformacao Tecnologica, Brasilia, ISBN-13: 9788573832839, pp: 404-459.

Lima, J.A.A., A.K.F.D. Silva, M.D.L. Aragao, N.R.D.A. Ferreira and E.M. Teofilo, 2011. Simple and multiple resistances to viruses in cowpea genotypes. Pesquisa Agropecuaria Brasileira, 46: 1432-1438.

Mali, V.R. and K.S. Kulthe, 1980. A seed-borne potyvirus causing mosaic of cowpea in India. Plant Dis., 64: 925-928.

Mali, V.R., F.S. Patil and D.H. Gaushal, 1981. Immunity and resistance to bean yellow mosaic, cowpea aphid borne mosaic and tobacco ringspot viruses in cowpea. Indian Phytopathol., 34: 521-522.

Naidu, R.A. and J.A. Hughes, 2003. Plant virology in sub-Saharan Africa. Proceedings of the Conference of International Institute of Tropical Agriculture, June 4-8, 2001, Ibadan, Nigeria, pp: 233-260.

Pio-Ribeiro, G., S.D. Wyatt and C.W. Kuhn, 1978. Cowpea stunt: A disease caused by a synergistic interaction of two viruses. Phytopathology, 68: 1260-1265.

Pio-Ribeiro, G., S.S. Pappu, H.R. Pappu, G.P. Andrade and D.V.R. Reddy, 2000. Occurrence of cowpea aphid-borne mosaic virus in peanut in Brazil. Plant Dis., 84: 760-766.

Rachie, K.O., 1985. Introduction. In: Cowpea Research, Production and Utilization, Singh, S.R. and K.O. Rachie (Eds.). John Wiley and Sons, New York.

Salem, N.M., J.D. Ehlers, P.A. Roberts and J.C.K. Ng, 2010. Biological and molecular diagnosis of seedborne viruses in cowpea germplasm of geographically diverse sub-Saharan origins. Plant Pathol., 59: 773-784.

Shoyinka, S.A., G. Thottappilly, G.G. Adebayo and F.O. Anno-Nyako, 1997. Survey on cowpea virus incidence and distribution in Nigeria. Int. J. Pest Manage., 43: 127-132.

Taiwo, M.A., 2003. Viruses Infecting Legumes in Nigeria: Case History. In: Plant Virology in Sub-Saharan Africa, Hughes, J.A. and B.O. Odu (Eds.). International Institute of Tropical Agriculture, Ibadan, Nigeria, pp: 365-380.