SEED TRANSMISSION OF COWPEA VIRUSES

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Abstract
A study was undertaken to identify the viruses present in commercial cowpea seedlots, compare their rates of seed transmission and assess the multiplication rates in subsequent generations in both local and improved cowpea varieties. A total of 222 cowpea seedlings were raised in the glass house of I.A.R &T, Moor plantation Ibadan. The seeds were obtained from Bodija market, Ibadan. About 108 leaf samples with virus-like symptoms were collected and assayed serologically in three different tests using agar gel double diffusion test and enzyme linked immunosorbent assay (ELISA). The six transmitted viruses were southern bean mosaic (SBMV)-sobemovirus, cowpea mottle (CMeV)-carmovirus and cowpea yellow mosaic (CYMV)-comovirus, for the agar gel test and cucumber mosaic (CMV)- cucumovirus for ELISA. The third test is also ELISA for the presence of potyviruses which is black eye cowpea mosaic (BICMV) and cowpea aphid borne (CABMV). Seed-borne viruses were detected in 14 out of 108 of the symptomatic plant samples. The viruses detected and the percent incidences were: BICMV 1.3 to 25% (20), CMV 5.26 to 6.25% (20), SBMV 1.85% (108). CBMV, CYMV and CMeV were not detected. Also, leaf samples were collected and tested for seed transmission from parent to offspring by ELISA-detectable seed-borne viruses. For potyvirus (BICMV & CABMV) from progeny (F_1) of nine cultivars that produces seed was re-sown out of the fourteen cowpea varieties. Only BICMV was transmitted through seed at 55.56% (5/9) frequency. No virus was detected in TVU 57 and Oloyin varieties.

Key words: Seedborne, Virus, Transmission, Cowpea, Offspring, Serology Assay and ELISA

Introduction

Cowpea (*Vigna unguiculata* (L) Walp) belongs to the family Leguminoseae, sub-family Papillionaceae of Genus Vigna. Other important legumes include: Soybean -*Glycine max*, Groundnut- *Arachis hypogeae*, Bambarra bean- *Voandzeia subterranea*.

It is an annual crop believed to have originated from Africa and grown in Nigeria and other parts of the world but especially cultivated troughout the lowland tropics and sub-tropics of Africa. Cowpea besides being important soil improving leguminous crop, is grown as a vegetable and also as food grain of high nutritive value like protein for human consumption and as fodder for animal.

In Nigeria, the planting time of this crop vary with cultivars and geographical location. In the savannah zone the late maturing varieties are planted in June as rain is steady, the medium maturing varieties are planted by middle of July and early varieties planted late July or first two weeks of August. In the forest zone, the late maturing varieties are planted in July, while the medinm varieties are planted early in August and the early varieties are planted by August ending.

Pest and diseases are known to infect crops at all stages of life of the plant. Virus is one of the disease that infect crops including cowpea in Nigeria (Phatak, 1974). Viral diseases have disastrous effect on crop yield and threaten the food producing potential of Africa and other parts of the world (I.I.T.A Research highlight, 1980).

Viral transmission of cowpea seeds are disseminated through infected seedlots sold commercially in the markets, and distributed over wide geographical and ecological region, when farmers uses those seeds (Shoyinka, *et al*., 1978).

Fourteen viral diseases were isolated in India, as seed borne out of about twenty-four viral diseases reported from different cowpea producing areas (Mali and Thottappilly, 1985). Namely: blackeye cowpea mosaic (BICMV), cowpea aphid-borne (CAbMV) and peanut mottle (PMV) as potyviruses; cumcumer mosaic (CMV) as cucumovirus, sunhemp mosaic (SHMV) as bamovirus, cowpea mild mottle (CMMV) and cowpea mottle (CMeV) as carmovirus, cowpea mosaic (CPMV) and cowpea severe mosaic (CSMV) as comoviruses, while southern bean mosaic (SBMV) belong to the sobemovirus group respectively (Shoyinka *et al*., 1979; Allen *et al*., 1981; Taiwo *et.al*., 1982; Bashir and Hamptom, 1993).

Virus identification based on host plant reaction alone is seldom reliable, therefore, serology test is required for the positive identification and confirmation of the different strains of viral diseases being isolated. Agar Gel Double Diffusion (AGDD), is a precipitate serology test for detecting virus disease involving the use of Agar plate and Antibody. The plate has wells,

containing different 'antigens' of crushed leaf samples tested for related antibody, when homologous reaction occur the test is positive and vise versa. Furthermore, a confirmatory test is needed with Enzyme Linked Immunosorbent Assay test (ELISA). This is a sensitive and accurate means for detecting numerous plant virus, and their potential application to crop germplasm resources. The present study was undertaken with six viral isolates, with the following objectives to:

1. Identify types of disease infection present in both local and improved varieties.
2. Compare the rate of seed transmission of the virus disease in the local and improved cowpea varieties in Nigeria.
3. Assess the multiplication rate of this virus in subsequent generation (F_2).

Materials and Methods

Sources of Seeds of Test Varieties

The present study was carried out with eight improved breeding lines from International Institute of Tropical Agriculture (I.I.T.A) and six local varieties of cowpea obtained from a commercial market (Bodija). The original sources of cultivation of the local cultivars by States were: Yola, Kano, Maiduguri, Yobe and Sokoto respectively.

Growing-on Test and observation of Diseaes Symptoms

Seeds of the test cowpea varieties were planted four seeds per pot in 2 litre plastic pots. Five pots werer planted to each test cultivar in the green house at Institute of Agricultural Research and Training, Ibadan for observation of virus symptoms.
The seedlings were examined for appearance of disease symptoms first at the primary leaf (cotyledon) stage and later whan the first trifoliate leaf became fully expanded. The number of seedlings showing viral disease symptoms were counted at weekly intervals. All plants were sprayed weekly with Monocrotophos i.e nuvacron (405CW a.i. 400 gl^{-1}) at the rate of 2 ml per litre of water to eliminate insect vectors and also with Brestan 60, a fungicide at $5gl^{-1}$ to control soil borne fungal Pathogens like Pythium fusarium karate (2.5EL a.i.$25gl^{-1}$) was seperatly sprayed at the rate of $1ml^{-1}$ of water to control Mealy bug infection.

Assessment of Second Generation (F_2) plants

The infection plants were allowed to grow to maturity before being harvested. The seeds from all infected plants of each variety were bulked and resown as F_2 seedlings in trays that contained heat sterilized soil, for growing-on test and observation for seed transmitted viral diseases.

Preparation of Samples for Serology

For virus identification, infected leaves and two healthy leaves from each cultivar were tested serologically. Extracts from tissue samples were grinded in appropriate buffers and tested either by Agar Gel Double Diffusion test (AGDD) or Enzyme -Linked Immunosorbent Assay (ELISA).

A. Agar – Gel Diffusion Test

(i) Preparation of Agar-gel: 2.25g agar and 2.13g sodium chloride was weighed and dissolved in 250ml distill water in a conical flask using a stirrer. Therafter, the solution was capped with aluminium foil and autoclaved at 121^oC / $1.1kgcm^{-2}$ for 40minutes; 0.4g Sodium azide (NaN_3) was added to the medium and allowed to cool. The content was then poured into sterile petric dishes (about 15ml each) on a level top whose surface has been sterilized with spirit in the inoculation room.

(ii) Agar- Gel Test Procedure: 0.25g of each of the diseased cowpea plant samples was weighed and grinded with 2.5ml of 0.1M pH 7.4Phosphate buffer solution (PBS) in a glass plate using a sterile pestle. Some cotton wool was used to absorb the sap and squeezed into glass tubes arranged in a rack.

The agar gel diffusion plates were loaded with virus antiserum. At the centre, the peripheral wells were loaded with sap dillutions of the samples and left at room temperature for 24hours. Only antisera to SMBV, CMeV and CYMV were used in this tests.

B. Enzyme – Linked Immunosorbent Assay

(1) Preparation of buffers

(ia) Phosphate buffer solution – Tween 20, pH7.8 (PBS-T) 8.0g NaCl x2; 0.2g KH_2PO_4 x2; 1.1g Na_2HPO_4 x2; 0.2g KCl x2; 0.2g NaN_3 x2 in 2litres of distill water.

(ib) Coating buffer (pH9.6) for 1litre – 1.59g Na_2CO_3 x0.5, 2.93g $NaHCO_3$ x0.5, 0.2g NaH_3 x0.5 in 0.5litre of distilled water.

(ic) Conjugate duffer – PBS-Tween containing 2.0g ovalbumin and 20g PVP litre^{-1} to inhibit non-specific binding of the conjugate.

(id) Substrate buffer (pH9.8) for 1litre-97ml diethano – lamine, 800ml distilled water, 0.2g NaN_3, add concentrated HCl to adjust to pH9.8 and bring up the volume to 1litre.

(ie) Blocking solution – PBS+10g Bovine serum Albumin litre^{-1} or 2.5% fat-free dried skimmed milk in PBS – Tween as a blocking agent.

(2) ELISA Test Procedure:

The monoclonal antibodies (Mab 15E6 for BICMV and MAB 5H5 for CAbMV) were diluted with coating buffer (1µ1 and 5µ1 per ml respectively). Each well was added 100µ1of each dillution and incubated for 2 hours at 37^{O}C. Excess antibody was decented and plates were washed with PBS – T (washing was done 3 times and for each wash PBS-T was left in the plates for 3 minutes). The plates were blocked for 30minutes at 37^{O}C using 2.5% MARVEL, 99%fat free dried skimmed milk in PBST as blocking agent.

The plant samples were grinded in PBS-T plus 2% PVP-40T (Polyvingl / Pyrrolidine) at a dilution, of 1:10. Each well was loaded with 100µ 1 of the sap dilution and incubated at 4^{O}C overnight.

In the morning, excess dilution were washed off as in step 2 above. The Mab- Biotin (10G5. Biot and 5H5 Biot for BICMV and CAbMV) were diluted in PBS-T buffer (1 µ1 for 10ml for the twoantibodies), loaded at 100 µ1per well and inoculated at 37^{O}C for 2 hours.

Excess antibody was decanted and washed as in step 2 above. A conjugate, containing streptomycin, alkaline prosphate in PBS-T (1:2000 dilution) and 2% PVP was prepared into each well, 100µ1 of the conjugate was added. The plates were incubated for 2 hour at 37^{O}C. The same procedure of washing with PBS-T as in step 2 was done , after 2hours.
The substrate buffer with its pH adjusted to 9.8 using HCl was prepared and ten tablets of the alkaline phosphatase was dissolved in 50ml of the solvent. Each well was loaded with 100 µ1 of the composition. The plates were obseved for a period of time. All wells with a colour change to yellow indicated presence of vius disease.

Results

The results indicated that 3 viral diseases: SBMV, BICMV and CMV were detected in 12 out of 14 varieties tested by serological methods (Plates 1,2 and 3). The relative incidence of infection of the 14 varieties observed by visual observation in (Table1), shows that TVU11952 Kanarnado has the highest percentage incidence rate of (75%) while TVU57 has the lowest (10%) .

Only a virus disease- SBMV was detected by Agar Gel Diffusion Technique in two different cowpea varieties, while CMV and BICMV were identified with three types of ELISA and CDNA probe positive identification. CMV was detected in cowpea cultivars TVU56 and Sokoto white at percentage infection rate occuring in 10 out of the 12 infected varieties, TVU11952 has the highest rate of infection (25%) followed by TVU11953 (16.67%) , Kuara (7.69%), TVU67 (6.25%), Drum (5.55%) , Sokoto White (5.26%) , TVU101 (5%), Olo (3.1%), TVU66 (1.9%) and L25 (1.3%) respectively (Tables 2 and 3). There was double infection of SBMV and BICMV on L25, likewise a mixed infection of CMV and BICMV on Sokoto white (Table 2).

Almost all the varieties identified to be infected by growing-on test in the glasshouse (Table 1 and Plates 1, 2 and 3). When subjected to different serological techniques 10 BICMV, 2 CMV; 2 SBMV and 2 different viral combinations, on 2 different cowpea varieties were identified respectively (Tables 2, 3 and Plates 1, 2 and 3).

Discussion

Serodiagnosis of cowpea virus has indicated the transmission of 3 viral diseases (SBMV, CMV and BICMV), belonging to 3 taxonomic groups: Poty, Cucumo and Sobemo, through the seeds of cowpea cultivars collected from Bodija market representing different places of cultivation , thus, pointing to their widespread distriution in Nigeria. However, the level of seed transmission of cowpea virus was found to vary with the different strains isolated, depending on virus-cowpea cultivar interaction. This agrees with the work of Aboul-Ata *et al.* (1982), Ladipo *et al*. (1979b), Mali *et. al*. (1989) etc.

Serological identification has shown that BICMV as the most prevalent vius disease with an incidence rate of 71.43% occuring in 10 cultivars out of 14 cowpea cultivars raised, while SBMV and CMV occurred in 2 cowpea cultivars each with incidence rate of 14.29%. Mixed viral infections have disastrous symptoms, causing more serious crop losses than it should have caused by a single virus, as observed on cowpea cultivars L25 (improved from I.I.T.A) and Sokoto White (local) variety collected from open market – Bodija and grown in the I.A.R &T glass house, all at Ibadan. This agrees with

the findings of Shoyinka, (1979), Bashir and Hampton(1993), when 108 leave samples were tested for SBMV-2 (1.85%) was detected, for CMV out of 20 samples 2(10%) was detected, and for BICMV out of 20 samples 10 (50%) were identified respectively.

Conclusions

Seed – borne disease virus was observed in local as well as improved cowpea varieties, such as CMV, SBMV and BICMV with high incidence rate of seed transmission to Blackeye Cowpea Mosaic Virus (BICMV) at a percentage infection rate of 16.67 to 25.00% for TVU11953 and TVU11952, Kaura and Mala at 5.55% and 7.69% respectively for F_1 generation (Table 2), while in subsequent generation (F_2) a seed transmission rate of 1.9% - TVU66, 2.5% - TVU67, 3.1% - Olo and 5.3% for Sokoto White was recorded.

CYMV, CMeV and CAbMV were not identified but this does not mean it is absent, however, one is limited with the number of cowpea varieties and the number of leave samples tested are very few.

Recommendations

From the results of the experimental work carried out the following are absolutely necessary-:

1. Increased cultivation of Oloyin (local) and TVU57 (improved) cowpea varieties by farmers so as to avoid or reduce the incidence of seed – transmitted viral diseases such as CMV, SBMV and BICMV.
2. These varieties can be used as breeding material, so as to reduce the occurrence of seed – borne infection through the development and use of pathogen – free sources of genotype collections of plant species (Williams, 1977; Ladipo *et al*., 1979b).
3. Roguing of infected plants at the onset of first symptoms to reduce disease incidence and spread since this serve as a source of primary inoculum.
4. Also, seed lots that is virus free could be stocked for commercial cowpea production in Nigeria.

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Table 1: Growing – on Test and Observation of Disease Symptoms

Varieties	No. Infected	No. Planted	Percentage (%) Incidence Rate
TVU67	4	16	25.00
TVU101	3	20	15.00
TVU11953 Karnanado	3	6	50.00
TVU66	4	15	26.66
TVU57	2	20	10.00
TVU11952Karnanado	3	4	75.00
TVU56	7	19	36.84
L25	3	20	15.00
Sokoto White	6	19	31.58
Olo	7	18	38.89
Drum	3	20	15.00
Kaura	5	15	33.33
Mala	8	20	40.00
Oloyin	7	19	36.84

Table 2: Results of Serology Test and Levels of Disease Incidence

Varieties	1st Assessment AGDD 13Days after planting	Percentage (%) infection	2nd Assessment ELISA test 17Days after planting CMV	Potivirus	Percentage (%) infection	Identified Viral Diseases
TVU67	0/16	0	0/16	1/16	6.25	BICMV
TVU101	0/20	0	0/20	1/20	5.00	BICMV
TVU11953 Karnanado	0/6	0	0/6	1/6	16.67	BICMV
TVU66	0/15	0	0/15	-	0	BICMV
TVU57	0/20	0	0/20	0/20	0	-
TVU11952Karnanado	0/4	0	0/4	1 / 4	25.00	BICMV
TVU56	0/19	0	1/16	0/16	6.25	CMV
L25	1/20	5	0/20	0/20	0	BICMV
Sokoto White	0/19	0	1/19	0/19	5.26	BICMV
Olo	0/18	0	0/16	0/16	0	BICMV
Drum	0/20	0	0/18	1/18	5.55	BICMV
Kaura	0/15	0	0/13	1/13	7.69	BICMV
Mala	1/20	5	0/20	0/20	0	SBMV
Oloyin	0/19	0	0/19	0/19	0	-

KEY: AGDD is the First Assessment involving SBMV,CYMV and CMeV Antisera using Agar Gel Double Diffusion test. ELISA test – Second Assessment using three types of ELISA and CDN probe. +ve identified, -ve not detected.

Table 3: Comparism of Seed Transmission Rate of some Cowpea Viral Diseases between the First and Second Filial Generation

Varieties	Incidence(%) F_1	Incidence(%) F_2	Transmission Rates in Percentage (%) $SBMVF_1$	$CMVF_1$	$BICMVF_1$	$BICMVF_2$
TVU67	25.00	27.50	-	-	6.25	2.5
TVU101	15.00	1.75	-	-	5.00	1.75
TVU11953 Karnanado	50.00	-	-	-	16.67	-
TVU66	26.66	26.42	-	-	-	1.9
TVU57	10.00	3.20	-	-	-	-
TVU11952Karnanado	75.00	-	-	-	25.00	-
TVU56	36.84	-	-	6.25	-	-
L25	15.00	48.75	5	-	-	1.3
Sokoto White	31.58	31.58	-	5.26	-	5.3
Olo	38.89	75.00	-	-	-	3.1
Drum	15.00	-	-	-	5.55	-
Kaura	33.33	20.00	-	-	7.69	-
Mala	40.00	-	5	-	-	-
Oloyin	36.84	76.00	-	-	-	-

Diseased leaf samples collected for Serology Test

PLATE 1: Mala-SBMV

PLATE 2: Sokoto White-BICMV

PLATE 3: TVU 56-CMV

At primary & trifoliate leave stages