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Research Application Summary

Seed-borne viruses detected on farm-retained seeds from smallholder farmers in Zimbabwe, Burkina Faso, Bangladesh and Vietnam

Manyangarirwa, W.[1], Sibiya, J.[2] & Mortensen, C.N.[3]
[1]Faculty of Agriculture and Natural Resources, Africa University, P.O. Box 1320, Mutare, Zimbabwe
[2]Faculty of Science and Agriculture, University of Kwazulu- Natal, P. Bag X01, Scottsville 3209, Pietermaritzburg, South Africa
[3]Danish Seed Health Centre, University of Copenhagen, Thorvaldsensvej 40, DK 1871, Frederiksburg C, Copenhagen, Denmark
Corresponding author: manyangarirwaw@africau.ac.zw

Abstract

The smallholder farming sector in much of the developing world relies on the use of farm-retained seed. The availability of good quality disease free seed is important in enhancing food security but seed-borne viruses can be a major problem on farm-retained seed. Seeds of tomato (*Lycopersicon esculentum* Mill.), paprika (*Capsicum annuum* L.), cowpea (*Vigna unguiculata* L. Walp), bambara [*Vigna subterranea* (L.) Verdc.] and peanut (*Arachis hypogaea* L.) from smallholder farmers in Zimbabwe, Burkina Faso, Bangladesh and Vietnam were tested for seed-borne viruses using various techniques including electron microscopy, Enzyme Linked Immunosorbent Assay (ELISA) and biological assays. Tomato mosaic virus (ToMV) was detected in 36% of tomato samples and in 8% of paprika samples using indicator *Nicotiana tabacum* cultivars Xanthi-nc and White Burley. Some 43% of cowpea samples were infected with Cowpea aphid-borne mosaic virus (CABMV) and 7% were infected with the Blackeye cowpea mosaic strain of Bean common mosaic virus (BCMV-BlCM). Peanut mottle virus (PeMoV) was detected with an infection range of 5.4%-12.5% in bambara samples tested using indirect antigen-first ELISA, indicator IITA cowpea lines Tvu 3433, Tvu 1582, Tvu 401 and Tvu 2657 and *Phaseolus vulgaris* cultivar Topcrop. No viruses were detected in the peanut samples. The lack of a broad range of serological antisera limited the number of viruses tested but results show that the bulk of the samples were infected with seed-borne viruses. The results imply that resources must be invested in improving the quality of seed from the smallholder sector. National and international germplasm collection centres should be equipped with capacity to test for seed-borne viruses so as to minimize the movement of infected germplasm in breeding materials or germplasm exchange in international collections.

Key words: Detection, farm-retained seed, smallholder farmers

Résumé

Le secteur des petites exploitations agricoles dans la plupart des pays en développement repose sur l'utilisation des semences retenues de ferme. La disponibilité de semences de qualité sans maladie est importante dans le renforcement de la sécurité alimentaire, mais les virus transmis par les semences peuvent être un problème majeur sur les semences retenues de ferme. Les semences de tomates (*Lycopersicon esculentum* Mill.), Paprika (*Capsicum annuum* L.*)*, le niébé (*Vigna unguiculata* L. Walp), bambara *[Vigna subterranea (*L.*)* Verdc.] et l'arachide (*Arachis hypogaea* L.) à partir de petits agriculteurs du Zimbabwe, du Burkina Faso, de Bangladesh et du Vietnam ont été testés pour le virus transmis par les semences en utilisant diverses techniques, y compris la microscopie électronique, l'immuno-enzymatique (ELISA) et des dosages biologiques. Le virus de mosaïque de Tomato (ToMV) a été détecté dans 36% des échantillons de tomates et dans 8% des échantillons de paprika en utilisant l'indicateur *Nicotiana tabacum Xanthi-nc* cultivars et blanc coloré. Quelque 43% des échantillons de niébé ont été infectées par le virus de la mosaïque du niébé transmis par les pucerons (CABMV) et 7% ont été infectés par la souche Blackeye niébé mosaïque de virus de la mosaïque commune de haricot (BCMV-BlCM). virus de la marbrure d'arachide (PeMoV) n'a été détectée avec une gamme d'infection de 5,4% -12,5% dans les échantillons testés à l'aide indirecte bambara antigène-et-unième ELISA, les lignes de signalisation niébé IITA Tvu 3.433, 1.582 Tvu, Tvu 401 et 2.657 Tvu et Phaseolus vulgaris cultivar de culture de base. Aucun virus n'a été détecté dans les échantillons d'arachide. L'absence d'un large éventail d'antisérums sérologique limité le nombre de virus testés, mais les résultats montrent que la majeure partie des échantillons ont été infectés par des virus transmis par les semences. Les résultats impliquent que les ressources doivent être investies dans l'amélioration de la qualité des semences par le secteur des petits exploitants. Les centres nationaux et internationaux de collecte du matériel génétique doivent être équipés avec une capacité de test pour les virus transmis par les semences de façon à minimiser le mouvement de matériels génétiques infectés dans les matériaux de reproduction ou échange de matériel génétique dans les collections internationales.

Mots clés: La détection, la ferme de semences conservées, les petits agriculteurs

Background

Virus diseases have disastrous effects on crop yields and threaten the food production potential of Africa, Asia and other developing

parts of the world. A number of virus diseases are seed-borne and this compounds field crop losses since seed-borne viral inoculum serves as the primary source of infection in the field (Thottappily, 1992). This paper reports on the incidence of seed-borne viruses detected on predominantly farm-retained seeds of tomato, paprika, cowpea, bambara and peanut obtained from smallholder farmers in Zimbabwe, Burkina Faso, Bangladesh and Vietnam.

Literature Summary

The availability of quality seed is one major challenge facing farmers in most developing countries and even in some developed countries (Mathur, 1995). It is estimated that in Sub-Saharan Africa, up to 95% of the crop area of traditional crops planted by smallholder farmers is planted using farm-retained seed (Muliokela, 1999).

Seed testing for seed-borne viruses is constrained by factors such as the lack of well trained personnel, lack of antisera and lack of specialized equipment thus compromising the quality of virus detection tests conducted (Lange *et al*., 1983). The objective of this study was to draw up an inventory of seed-borne viruses on farm-retained seeds from different developing countries and to chart research priorities on seed-borne viruses in developing countries.

Study Description

One hundred and fourteen (114) farm-retained samples that included 53 of tomato, 12 paprika, 14 cowpea, 16 bambara and 19 peanut were tested for seed-borne viruses. The samples were kept in storage at 5^0C at the Seed Health Centre (SHC) in Denmark.

a) Testing for Tobamoviruses in tomato and paprika samples. An extract of 0.3 grams from each sample was gently rubbed in 10 ml of de-ionised water containing carborandum powder. The liquid extracts were used to inoculate indicator *Nicotiana tabacum* cultivars Xanthi-nc and White Burley. Negative controls were inoculated with de-ionised water. The plants were kept in a growth room at 20-25^{0}C and observed as from four days to 20 days after inoculation.

b) Growing-on test for seed-borne viruses in legumes. Two hundred (200) seeds per sample of bambara and cowpea were sown in pots with peat soil. The plants were maintained under artificial light with a photoperiod of 12-16 hours in growth rooms and normal daylight in the glasshouse at a temperature

of 25-30^{0}C. Seedlings were observed for virus symptoms from 2 weeks after planting until 6 weeks. Sap extracts from plants showing virus-like symptoms were examined using a transmission electron microscope (TEM) (model JEOL, JEM-100SX) and indirect ELISA. Sap extracts from asymptomatic seedlings were also examined to detect latent infections.

c) Sample preparation for Transmission Electron Microscopy. A piece of plant tissue (1-2 cm^2) from a symptom-bearing leaf was placed in a polythene bag with 1 ml of ammonium molybdate (AM), 2% in water adjusted to pH 7.0. The tissue was gently macerated and a drop of the extract was further diluted with drops of ammonium molybdate. The homogenate was mounted on carbon coated copper grids and examined in a JEOL, JEM-100SX transmission electron microscope.

d) Enzyme-linked immunosorbent assay (ELISA). Sap extracts from virus infected seedlings and showing virus particles in TEM were subjected to ELISA using the procedure developed by Hobbs *et al*. (1987). The ELISA plates were initially coated with 100 µl crude sap from suspected virus-infected plants in duplicate wells. Two wells were loaded with positive control samples and six wells were loaded with negative control samples. The following antisera were used: *Blackeye cowpea mosaic virus* (BlCMV-6) from Dijkstra, The Netherlands, isolate BlCMV-W from L. Bos, The Netherlands and BlCMV-8 (isolate H2) provided by IITA, Nigeria; *Bean common mosaic virus* (BCMV-11) isolate from F. Morales, CIAT, Colombia; *Cowpea aphid-borne mosaic virus* (CABMV-5) provided by K. Bock, Kenya and CABMV-9 isolate H4 from IITA; *Peanut mottle virus* (PeMoV-6) supplied by D.V.R. Reddy, ICRISAT and *Peanut stripe virus* (PStV-2) Indian isolate provided by D.V.R. Reddy, ICRISAT. Antisera were cross-absorbed with sap extracts from healthy plants of the homologous host prior to use. Swine-anti rabbit IgG, conjugated with alkaline phosphatase (DakoCytomation^{TM}) was used with p-nitrophenyl phosphate in diethanolamine buffer as substrate. Absorbance was read on an ELISA reader at a wavelength of 405 nm. Cowpea plants that were positive in ELISA were tested by inoculation on indicator plants *Phaseolus vulgaris* cv. Topcrop, *Vigna unguiculata* lines Tvu 1582 and Tvu 401 indicative of BlCMV, Tvu 2657 and Tvu 3433 indicative of CABVM. The indicator plants were checked for symptoms two days after inoculation up to 21 days.

e) Test for *Arachis hypogaea* viruses. Eighty small, shriveled dry seeds per sample were crushed in antigen buffer and tested by ELISA for the presence of *Peanut mottle virus* (PeMoV) and *Peanut stripe virus* PStV.

Research Application

Local lesions observed on *N. tabacum* cv. Xanthi-nc indicated the presence of Tomato mosaic virus (ToMV) in tomato and paprika seeds from Zimbabwe and Bangladesh (Fig. 1). The lack of systemic symptoms on the cultivar White Burley indicated the absence of Tobacco Mosaic Virus (TMV) infection. Cowpea aphid borne mosaic virus (CABMV) was detected in some cowpea samples from Zimbabwe, Burkina Faso and Vietnam (Table 1) and filamentous particles were observed in Electron Microscopy. Blackeye cowpea Mosaic Virus (BlCMV) was detected in one cowpea sample from Vietnam (Table 1). Peanut mottle virus (PeMoV) was detected in some bambara samples from Zimbabwe (Table 2). In extracts from plants with virus-like symptoms examined in electron microscopy, only filamentous particles were observed. Sap from plants that reacted with PeMoV, also induced symptoms on *P. vulgaris* cv. Topcrop within 3 days post inoculation, confirming the presence of the virus. For all peanut samples tested, the target viruses Peanut mottle virus and Peanut stripe virus were not detected.

Table 1. Growing-on and serological tests on cowpea seeds from Zimbabwe, Burkina Faso and Vietnam.

Accession	Country	% infected seedlings	Symptoms	Virus(es) detected in ELISA
46172	Zimbabwe	5.6	Stunting, chlorosis mosaic/mottle	CABMV
44821	Burkina Faso	1.5	CABMV	
44824	Burkina Faso	10.3	CABMV	
44825	Burkina Faso	0.5	Vein clearing, mosaic	CABMV
45510	Vietnam	0.6	Leaf down-folding	BlCMV
		2.2	Chlorotic spots, mosaic	CABMV

Table 2. Growing-on and serological tests for bambara seed-borne viruses on samples from Zimbabwe.

Accession	% infected seedlings	Symptoms	Virus detected in ELISA
40988	8.2		PeMoV
40989	12.5		PeMoV
41723	6.7		PeMoV
46166	12.5	Down-folding and	PeMoV
46167	6.3	narrowing of leaves,	PeMoV
46168	8.2	leaf mosaic	PeMoV
46169	8.8		PeMoV
46170	5.4		PeMoV

Figure 1. Local lesions on leaves of *Nicotiana tabacum* cv. Xanthi-nc used to detect Tomato mosaic virus (ToMV) in tomato and paprika seeds from Zimbabwe and Bangladesh.

Recommendation

The practical application of testing for seed-borne viruses is so far restricted to a few programmes. This implies that much of the seed traded between different countries does not pass through screening for seed-bore viruses and hence efforts to stem the movement of seed-borne viruses of quarantine importance are greatly compromised.

In most quarantine stations, the growing-on test is the most widely used technique for the detection of seed-borne viruses or sap borne viruses in the case of vegetatively propagated material (Thresh, 1998). One major limitation of the growing-on test is that it relies heavily on visual judgement and chances of erroneous interpretation are invariably greater. This implies that a number of virus detection techniques have to be employed at the same time and in some occasions in order to confirm one virus only. Results from the study suggest that in order to reduce the levels of infection in farmers' seeds and germplasm collections, funding has to be invested in improving the overall seed production systems because, besides the threat of seed-borne pathogens, the use of farm-retained seed also presents drawbacks such as low seed viability and loss of genetic purity.

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