BIOTECHNOLOGY AND BIOLOGICAL SCIENCES RESEARCH COUNCIL &
DEPARTMENT FOR INTERNATIONAL DEVELOPMENT

FINAL REPORT FORM

SECTION 1: PROJECT DETAILS

Grant Reference	BB/F004028/1	**Capacity Building Grant Reference**	BB/H531743/1
Project Title	How resistant plant varieties avoid suppression of RNA silencing by viruses as exemplified by sweetpotato: Better food security through virus control		
Principal Investigator	Dr Richard W Gibson		
Lead Research Organisation & Department	University of Greenwich, Natural Resources Institute		

Co-Investigator(s)	**Research Organisation**
Dr Settumba Mukasa	Makerere University, Uganda
Mr Peter Wasswa [PhD student]	UoG /Makerere University
Prof Sir David Baulcombe	University of Cambridge, UK
Dr Betty Owor	Makerere University/ University of Cambridge
Dr Jan Kreuze	International Potato Center [CIP], Peru
Dr Wilmer Cuellar	International Potato Center [CIP], Peru
Dr Neil Boonham	Food & Environment Research Agency [FERA], UK
Dr Gorrettie Ssemakula	National Crops Resources Research Institute [NaCRRI], Uganda

SECTION 2: PROJECT PROGRESS

Original objectives of research with proposed timescales for each objective	**Progress – <u>in the past 12 months</u> - against objectives and milestones.** If the project has suffered any setbacks, please indicate what, if any, remedial action will be taken and whether, and to what extent, the outcome of the project will be affected.
1. **Biological characteristics of sweetpotato/virus interactions, achieving a broad**	Analysis of the sequence of the hsp70 region suggested the occurrence of a third strain of SPCSV in central southern Africa named SA strain. Interestingly, sequence characterization of the coat protein region of SPCSV isolates from Africa and the Americas did not separate the West African and East African groups. Complete genomes of two new cavemoviruses named Sweet potato caulimo-like

overview. Timescale: Duration of grant	virus (SPCV) and Sweet potato vein clearing virus (SPVCV) have been obtained and their variability studied. The cavemoviruses were obtained from Central America, Caribbean islands and East Africa. Detection of pararetroviruses had been difficult in sweetpotato in the past because they are symptomless, difficult to purify and no sequence was available. A purification method has been developed and two pararetroviruses have been sequenced and primers for detection by PCR have been tested and published. A new sweet potato carlavirus previously known as C-6 has been characterized. It has not yet been reported in Africa and is only 55% identical in amino acid sequence to *Sweet potato chlorotic fleck virus* (SPCFV) a previously characterized sweet potato carlavirus found in Africa. SPCV, SPVCV, the new pararetroviruses and C-6 are all synergized by SPCSV; that of SPCV and SPVCV being the first synergistic interaction recorded between a DNA and an RNA virus. However, four sweepovirus isolates obtained from East Africa and the Americas were not associated with more severe symptoms in sweet potato in co-infection with SPCSV, agreeing with Wasswa et al. (2011). Curiously, however, a fifth isolate (collected in St Vincent Island) was synergised, inducing leaf curling symptoms in sweet potato (cv. Huachano) in co-infection with SPCSV, begging the question 'What is the difference?' All potyvirus isolates closely related to (SPFMV) contain an extra overlapping gene in the P1 region of the potyviral polyprotein except Sweet potato latent virus. The function of this gene is not yet known.
2. **Increased knowledge of RS-based resistance in sweetpotato.** Timescale: Duration of grant	The breadth of RS-based resistance in sweetpotato is exemplified by its breakdown against diverse viruses during synergistic co-infection with SPCSV (see above). RS-based resistance in sweetpotato is broken by the RNase3 gene of SPCSV, e.g., transforming sweetpotato with RNase3 results in plants which develop SPVD when infected with SPFMV alone. The p22 gene exacerbates it. Therefore, the model plant *Arabidopsis thaliana* was transformed with the RNase3 gene, with an alanine mutant RNase3 gene that has lost its ability to cleave siRNA (a control for the RNase3 mutation) and with the p22 gene. Few transformants with p22 survived but several of those of RNase3 and its Ala mutant survived. SPFMV infected neither untransformed controls nor the transformants but *Tobaco rattle virus* (TRV), *Turnip crinkle virus* (TCV) and *Cucumber mosaic virus* (CMV) all did. Both RNase3 and its mutant Ala were more susceptible to TRV and CMV than the parent untransformed line; RNase3, its mutant Ala and the parent untransformed line were all susceptible to TCV. We are seeking confirmation that the RNase3 was expressed in the two lines. If confirmed, it may be that cleavage of siRNA by RNase3 is not key to its role in synergy. The mild strain of SPCSV which synergised SPFMV in sweetpotato only poorly had an RNase3 gene and a p22 gene that closely resembled ones reported in the virus gene base for strongly synergising isolates of SPCSV. We are planning an experiment to examine whether the RNase3 gene is less expressed by the mild, poorly-synergising, strain than by strongly synergising wild type SPCSV.
3. **Is resistance to SPCSV in sweetpotato an extreme of RS-based resistance or otherwise?** Timescale: Duration of grant	A so-called mild strain of *Sweet potato chlorotic stunt virus* (SPCSV) from Busia, Uganda, has been shown to differ from wild type mainly in being able to synergise *Sweet potato feathery mottle virus* (SPFMV) in sweetpotato only poorly and seldom co-infecting. It provides only limited cross-protection against wild type, evidence that RS-based resistance has been switched off; yields of plants only infected with mild SPCSV are still halved so cross protection was not a useful attribute anyway. A 'hairpin' silencing construct targeting the 3'UTR of SPCSV, which is a low targeted genomic region by siRNAs in natural infection, was used to transform the Peruvian sweetpotato variety Huachano. SPCSV-infected plants returned to healthy status and no SPCSV was detected when indexing these plants in I. setosa. In plants inoculated with SPCSV + SPFMV (which would normally result in SPVD), SPCSV titre was dramatically reduced and SPFMV titers returned to those occurring in a SPFMV-single infected plant

4. Are sources of resistance effective in the field and their deployment? Timescale: Duration of grant	Varieties of sweetpotato such as New Kawogo that revert to healthy, probably through RS-based resistance, have less SPFMV when infected with SPFMV (or SPFMV + mild SPCSV) than varieties which seldom revert, such as Beauregard. This has been shown in glasshouse experiments in the UK using QPCR and in a screenhouse in Uganda using ELISA. As well as being consistent with RS-based resistance, it may provide the basis for a screening method for resistant varieties. NASPOT 11 [Tomulabula], a variety bred by participatory plant breeding and which resists virus infection is gradually being deployed in Uganda. A film was made of its selection process http://www.nri.org/work/tomulabula.html . Work on developing varieties resistant to SPCSV based on the resistance of DLP 3163 has stopped since it has been shown to be susceptible in Uganda (see previous reports).
5. Train developing country partners in the use of deep sequencing (Capacity building grant). Timescale: 2010 onwards – See **Training**.	
Setbacks: It has proved impossible for Dr Wilmer Cuellar to obtain a visa to enter UK, despite repeated attempts. As a result, the work on transformed Arabidopsis had to be done by Dr Betty Owor, taking her from other work, and the benefits to her work from direct linkages with Dr Cuellar were not obtained.	

SECTION 3: OUTPUTS

Research products (e.g. methodologies, techniques, tools and resources)

Research methods in use by developing country scientists during the reporting period:

1. Quantitative polymerase chain reaction (QPCR) assay
2. Archiving gene databases
3. Deep sequencing [Using siRNA particles and high performance computers to compute viral sequences]
4. Double antibody and triple antibody enzyme linked immunosorbent assays (DAS & TAS ELISA)
5. In situ hybridization, northern blots, western blots, agrobacterium-mediated plant transformation

QPCR, as a marked improvement of PCR, has been introduced both to NRI, Namulonge Research Institute and Makerere University. Use of QPCR is a result of external grants enabling the purchase of the equipment by the new technology centre developed there. Peter Wasswa (SARID PhD student) played a key role in providing backstopping.

DAS and TAS ELISA for detection of SPFMV has been developed at Namulonge Research Institute and Makerere University. It is hoped that this technology will support the identification of resistant genotypes of sweetpotato

Deep sequencing: see **Training**

Agricultural technologies of relevance to the poor

The new variety, NASPOT 11, otherwise known as Tomulabula, was released in Uganda in late 2010. This variety was bred by a participatory approach which is particularly appropriate for developing varieties for subsistence farmers. Tomulabula has numerous characteristics which confirm this, e.g., suitability for piecemeal harvesting, drought resistance, possessing roots which are developed deep underground protected from weevils and, of particular relevance to this project, the ability to maintain itself free from infection with viruses particularly SPFMV.

A method is being researched for the identification of sweetpotato varieties with an ability to maintain themselves free from infection with viruses particularly SPFMV using ELISA and QPCR to detect plants which, when infected, have a low virus titre.

New scientific collaborations involving developing country partner(s) established during the reporting period

International collaboration for education and advanced level and PhD education grant.

Swedish University of Agricultural Sciences, Uppsala, Sweden. Collaboration established with the group of Dr. Eugene Savenkov from the Department of Plant Biology and Forest Genetics. Uppsala BioCentre, Uppsala, Sweden. 2011. The grant covers the total costs (housing, perdiem and airtickets) during 1 month for Dr Cuellar and one Peruvian student. Collaboration in sweet potato C-6 carlavirus and sweet potato C-9 cavemovirus characterization.

Publications - production of research information

Publications in peer reviewed international journal

Cuellar, W.J., De Souza, J., Barrantes, I., Fuentes, S., Kreuze, J.F.. **2011**. Distinct cavemoviruses interact synergistically with Sweet potato chlorotic stunt virus (Genus Crinivirus) in cultivated sweet potato. *Journal of General Virology* **92**:1233-1243.

Cuellar, W.J., Cruzado, K.R., Untiveros, M., Fuentes, S., Soto, M., Kreuze, J.F. **2011**. Genome characterization of a Peruvian isolate of Sweet potato chlorotic stunt virus (SPCSV): further variability and a model for p22 acquisition. *Virus Research* **157**:111-115.

De Souza, J., Cuellar, W.J. **2011**. Sequence analysis of the replicase gene of 'sweetpotato caulimo-like virus' suggests that this virus is a distinct member of the genus Cavemovirus. *Archives of Virology* **156**:535-537.

Gibson, R.W., Mpembe, I., Mwanga, R.O.M., 2011. The role of participatory plant breeding as exemplified by the release of the sweetpotato variety NASPOT 11 in Uganda in 2010. *Aspects of Applied Biology* **107**, Systems improvements to crop improvement, pp7 -76.

Mwanga, R.O.M., Niringiye, C., Alajo, A., Kigozi, B., Namakula, J., Mpembe, I., Tumwegamire, S., Gibson, R.W. & Yencho, G.C. 2011. "NASPOT 11", a sweetpotato cultivar bred by a participatory plant-breeding approach in Uganda. *Hortscience* **46**: 317-321

Gibson, R.W., Mpembe, I., Mwanga, R.O.M., 2011. Benefits of participatory plant breeding (PPB) as exemplified by the first-ever officially released PPB-bred sweet potato cultivar. *Journal of Agricultural Science* DOI:10.1017/S0021859611000190

P. Wasswa, B. Otto, M. N. Maruthi, S. B. Mukasa, W. Monger and R. W. Gibson 2011. First identification of a sweet potato begomovirus (sweepovirus) in Uganda: characterization, detection and distribution **Plant Pathology** DOI: 10.1111/j.1365-3059.2011.02464.x

Papers read:

Mwanga, R.O.M., G. Ssemakula, C. Niringiye, S. Tumwegamire, Yencho, C. and Gibson R.W. 2010. Release of NKA 1081L, the first sweetpotato cultivar in Sub-Saharan Africa developed using PPB approach. Presented at: 11th International Society of Tropical Root Crops – African Branch Symposium, in Kinshasa, DR Congo, October 4-8, 2010

Gibson, R.W., Mpembe, I., Mwanga, R.O.M., 2011. The role of participatory plant breeding as exemplified by the release of the sweetpotato variety NASPOT 11 in Uganda in 2010. Presented at: Association of Applied Biologists at Rothamsted, 14-15 April 2011.

De Souza, J., Fuentes, S., Gálvez, M., Kreuze, J., Cuellar, W.J. 2011. Genome characterization of two new cavemoviruses infecting sweet potato (*Ipomoea batatas* (L.) Lam.) and it synergistic interaction with *Sweet potato chlorotic stunt virus* (SPCSV). (*In Spanish*). Presentations at the XXX Colombian and XVI Latinamerican Congress of Phytopathology

Flores, M., Silvestre, R., Kreuze, J., Cuellar W.J. 2011. Evaluation of virus indexed plant material by deep

sequencing. (*In Spanish*) Presentations at the XXX Colombian and XVI Latinamerican Congress of Phytopathology

Cuellar, W.J. 2010. Advances in the characterization of viruses affecting root and tuber crops. Presented at the International Symposium: Plant Breeding in Postgenomic Era: Trends and Perspectives. November 23rd. Bogota, Colombia

Film:
Richard Gibson & Isaac Mpembe. Tomulabula: The first ever sweetpotato variety bred by participatory plant breeding in Uganda. http://www.nri.org/work/tomulabula.html

All authors who are from a developing country are in red

Infomedia – requests for research information (infomedia is the means used to share knowledge, e.g. newspaper, TV, radio, mobile phones, websites, magazines). This indicator is about measuring outreach in line with spend on research communications. It measures the **requests** for research information as opposed to the production of research information.

Record any infomedia you have used during the reporting period to disseminate information directly related to this project.

Data	**Number & Details**
Keyword/thematic area/headline statements from research appearing in infomedia	Total number of places where the keyword/thematic area/headline statements appears **as a result of requests**
actual radio interviews	NASPOT 11 (Tomulabula) was briefly mentioned in local Ugandan newspapers. Dr Gorrettie Ssemakula is planning to write a more extensive article on it.
actual television interviews	
actual features in newspapers, magazines, other similar publications	
infomedia websites that provide links to research programme[1]	2
Other - please state	A film at: **http://www.nri.org/work/tomulabula.html**

Training (knowledge/skills-based short courses; PhD; research fellowships; workshops; exchange visits; mentoring; knowledge-based networks/communities of practice; on the job training; self-study/guided reading)

A course on Deep sequencing data analysis for virus identification and characterization was provided at Makerere University, Kampala, Uganda. May 2011. The course consisted of Laboratory training in plant RNA purification and computer sessions on data analyses and visualization of results.

Participants: 6 students from Makerere University, 1 Postdoctoral fellow from NRI, 1 postdoctoral fellow from Cambridge University, 1 Postdoctoral fellow from Mikocheni Laboratory, 1 postdoctoral fellow from Makerere University, 2 Graduate students from NaCRRI. *[Capacity building grant BB/H531743/1]*

Mentoring
Peter Wasswa. NRI, UK and Makerere University, Uganda. Graduate thesis work in progress: Sweet potato viruses in Uganda: identification of a new virus, a mild strain of an old virus and reversion.

Joao De Souza. Universidad Nacional Agraria. Lima, Peru. Undergraduate thesis completed:

Characterization of a new pararetrovirus from sweet potato. Graduate thesis work in progress: Characterization of viruses infecting root and tuber crops in South America.

Marco Gálvez. Universidad Nacional Agraria. Lima, Peru. Graduate thesis work: Sweepoviruses and their interactions with Sweet potato chlorotic stunt virus (*Crinivirus*, SPCSV).

Mirella Flores. Universidad Nacional San Martin, Lima, Peru. Graduate thesis work in progress. A bioinformatic protocol for the early characterization of emerging viruses.

International collaboration for education and advanced level and PhD education grant.

Swedish University of Agricultural Sciences, Uppsala, Sweden. Collaboration established with the group of Dr. Eugene Savenkov from the Department of Plant Biology and Forest Genetics. Uppsala BioCentre, Uppsala, Sweden. 2011. The grant covers the total costs (housing, per diem and airtickets) during 1 month for Dr Cuellar and one Peruvian student. Collaboration in sweet potato C-6 carlavirus and sweet potato C-9 cavemovirus characterization.