BIOTECHNOLOGY AND BIOLOGICAL SCIENCES RESEARCH COUNCIL &
DEPARTMENT FOR INTERNATIONAL DEVELOPMENT

ANNUAL 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 [UoG] Natural Resources Institute [NRI]		

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 Ian Barker	International Potato Center [CIP], Kenya Office
Dr Jan Kreuze	International Potato Center [CIP], Peru
Dr Wilmer Cuellar	International Potato Center [CIP], Peru
Dr Wendy Monger	Food & Environment Research Agency [FERA], UK
Dr Gorrettie Ssemakula	National Crops Resources Research Institute [NaCRRI], Uganda

Total Grant Value (£)	**Start Date**	**End Date**	**Original duration of grant (months)**	**Extension (as agreed with BBSRC Office)**
£652,539.47	11 August 2008	11 August 2011	36 mths	
Capacity building grant:				
£18,654	10 May, 2010	10 Aug, 2011		

Original objectives of research with proposed timescales for each objective (including any agreed amendment)

Original objectives:
RNA silencing (RS) through small interfering RNA (siRNA) is a main defence against viruses in all plants. Viruses also have means of suppressing RS and RS-based resistance is achieved only when suppression is avoided. Unlike vector based resistance, RS-based resistance is effective against systemic infections that can otherwise build up in vegetatively propagated crops. Such crops [cassava, sweetpotato, yams, bananas etc] are important in many developing countries -they dominate agricultural

production in most of Africa, especially by poorer people. In developed countries, certification schemes protect commercial cultivars from degeneration caused by viral infections but such schemes are not viable for sweetpotato and other vegetatively-propagated staple food crops grown in low-input, developing country systems. Instead, only cultivars that do not degenerate can thrive, e.g. landraces infected by *Sweet potato feathery mottle virus* (SPFMV), the commonest virus of sweetpotato worldwide, develop only mild symptoms followed by recovery and reversion to healthy. These are hallmarks of RS; cassava mosaic resistant cassava reacts similarly. Instead, the main viral disease of sweetpotato in Africa is sweet potato virus disease (SPVD), induced by co-infection of SPFMV with the crinivirus, *Sweet potato chlorotic stunt virus* (SPCSV); similar severe diseases also occur when SPCSV co-infects with other sweetpotato infecting viruses which cause only mild or no disease by themselves. RNA1 of SPCSV encodes two unique proteins, p22 and its own RNase3, which have been shown to suppress the host plant's RS-based resistance, 'freeing' co-infecting SPFMV to multiply uncontrollably. Because of this molecular evidence that RS may be the basis of one form of natural resistance to SPFMV, because sweetpotato genotypes with both genetically-engineered silencing and natural sources of a range of resistances including extreme resistance to SPFMV and SPCSV are available [not all of which may be RS-based] and because SPCSV provides a tool for suppressing resistance, sweetpotato provides a model crop for RS research. At the same time, sweetpotato is a vital crop in developing countries, especially for the more vulnerable. Susceptibility to viruses has delayed genetic improvement of sweetpotato in Africa, massively delaying deployment of high vitamin A orange-fleshed varieties. This project has two underlying themes: Increased understanding of how RS-based resistance functions and its better exploitation to protect vegetatively-propagated crops, and; Increased production of sweetpotato in Africa. Objectives to be achieved directly by the project are:

1. To identify the scope of natural RS-based resistance to SPFMV in sweetpotato, the range of viruses affected, its likely durability and effectiveness in the field.
2. To understand at the molecular level how RS-based resistance operates in sweetpotato sufficient to identify and use molecular markers of resistance in breeding programmes in Africa.
3. To assess the range of viruses and host plants for which RS-based resistance is suppressed by co-infection with SPCSV.
4. To determine whether different sources of resistance to SPCSV and to SPFMV are all RS-based and, if necessary, to understand how RS and non-RS may be integrated in practice.
5. To incorporate RS and novel S American sources of resistance to SPFMV and SPCSV in East African breeding programmes including for the orange-fleshed trait, through partnership with the Ugandan sweetpotato programme based at the National Crops Resources Research Institute (NaCRRI). This includes participatory breeding approaches to target poorer people.
6. To enable the molecular understanding of RS-based resistance to be grounded in the region through links with Makerere University so that it can be extended to other crops/situations. Inclusion of developing country research assistants and a PhD student will further ensure technology transfer*.

Additional Capacity Building Grant:

- April 1st 2010 [closing date]: Applications by Dr Settumba Mukasa, Dr Betty Owor, Dr Wilmer Cuellar and Mr Peter Wasswa for **Next Generation Sequencing** course
- 18-24 July 2010: Attendance at **Next Generation Sequencing** course
- 26-27 July 2010: Attendance at Cambridge University Department of Plant Sciences R/Bioconductor course
- August 2010: Installation of computer, office and bioinformatics software
- October 2010 onwards: Inclusion of Bioinformatics in Makerere University Agriculture course work

*Objective 6 is main objective to which Capacity building grant relates

Technical summary (as in original application)

RNA silencing (RS) is a fundamental plant defence involving small interfering RNA; RS-based resistance is achieved only when viral suppression is avoided. *Sweet potato feathery mottle virus* (SPFMV), the commonest virus of sweetpotato, induces only transient mild symptoms and associated reversion to healthy, hallmarks of RS defence, provides an alternative to certified virus-free schemes in low-input, developing country farming systems for sweetpotato and other vegetatively-propagated crops, e.g., cassava mosaic-resistant cassava. Sweetpotato virus disease (SPVD), the main disease of sweetpotato in Africa, involves both *Sweet potato chlorotic stunt virus* (SPCSV) and SPFMV. During co-infection, SPFMV increases in titre, often by several orders of magnitude and apparently in all tissues. SPCSV and SPFMV have already been sequenced. SPCSV has two RNA molecules: two proteins encoded by its RNA 1, p22 and an RNase3 have together been shown to suppress RS, providing a mechanism whereby SPCSV co-infection releases SPFMV from RS-based resistance and causes SPVD. How plants resist viruses through RS will be investigated by studying the known RS system in sweetpotato against SPFMV both in circumstance where resistant sweetpotato resist SPFMV when infecting alone and where it breaks down when co-infecting SPCSV suppresses RS. Diverse germplasm including extreme resistance to SPCSV and SPFMV now identified in CIP's worldwide sweetpotato collection, SPVD-tolerant African landraces and engineered resistance provide additional research entry points. Sweetpotato is a vital food and nutritional crop in many developing, especially African countries; partners include an African (Uganda) national breeding programme and university. The range and durability of SPFMV RS-based plant resistance, combining ability with other forms of resistance and molecular markers of resistance will be assessed, aiming to achieve rapid deployment of superior resistant varieties and sustainable control

SECTION 2: PROJECT PROGRESS (up to 2 x A4 pages)

Introduction: The project seeks both a broad understanding of resistance in sweetpotato against the main viruses infecting it in Africa and more specifically the resistance to *Sweet potato feathery mottle virus* [SPFMV] and how *Sweet potato chlorotic stunt virus* [SPCSV] breaks this resistance to synergise SPFMV and other viruses in sweetpotato. SPCSV was known to synergise SPFMV by blocking sweetpotato's virus resistance based on gene silencing: a greater understanding of this would allow this apparently broad-based resistance to be exploited better by plant breeders. The **Case for Support** compresses the six objectives listed in the **Objectives** into 4 main themes and, as in the 2008/9 Interim Report, these are reported against:

1: Biological characteristics of sweetpotato/virus interactions, achieving a broad overview: Preliminary results were reported in 2008/9 of the identification of a sweetpotato begomovirus [sweepovirus] for the first time in Uganda and the second time in Africa. The first full sequence of an African sweepovirus has now been obtained from Uganda. It appears to be a new virus and, like other sweepoviruses, it has a monopartite genome. Sweetpotato begomovirus isolates have also been found in Tanzania and reconfirmed in Kenya. This and our work with sweetpotato begomoviruses found in South and Central American countries have been brought together with other sequences in databases to show how sweetpotato begomoviruses separate into groups, with most East African samples falling within the 'Leaf curl' group.

An isolate of SPCSV giving negligible symptoms in the indicator plant *Ipomoea setosa*, purpling in sweetpotato and seldom being co-infected in the field with SPFMV [and therefore not with the severe symptoms of SPVD] has been found commonly in Kampala white, a popular cultivar in Eastern Uganda. Farmers indicated in interviews that affected plants provide poor planting material; yield trials are in progress to confirm this. Graft inoculations of typical wild type SPCSV ($SPCSV_{wt}$) to plants of cv Kampala white infected with this mild SPCSV ($SPCSV_{mld}$) failed to demonstrate potentially useful cross-protection between the two isolates; field trials are again in progress to confirm this.

The *Caulimoviridae* are a poorly studied virus group generally but perhaps especially the *Cavemovirus* genus and its species infecting sweetpotato. We have now sequenced the genomes of the two known cavemoviruses infecting sweetpotato worldwide and given them the acronyms SPCV-A and SPCV-B. Their genomic organisation resembles that of other *Caulimoviridae*, with 4 open reading frames; phylogenetic analysis shows they are most closely related to *Cassava vein mosaic virus*, a cavemovirus found in Brazil. Their means of transmission was not identified; aphids did not transmit them. The presence of SPCV-B as well as SPCV-A has been shown for the first time in Africa [Uganda & Kenya].

Deep or Next Generation sequencing is being successfully developed as a general method to detect latent, low titre sweetpotato viruses as well as for studying siRNAs. As well as detecting the common SPFMV and SPCSV, sequences corresponding to a new strain of SPFMV and isolates of cavemo-, begomo- and badnaviruses have been found in samples from Tanzania; the badnavirus appears to be a new species.

2: Increased knowledge of RS-based resistance in sweetpotato: The breadth of RS-based resistance in sweetpotato as exemplified by its breakdown against diverse viruses during synergistic co-infection with SPCSV and the mechanism has been studied. A S. American isolate of SPLCV was shown to be synergised by SPCSV and plants of infected Ugandan landraces frequently reverted to healthy, both signs of RS-based plant resistance. SPCV-A and SPCV-B have also been shown to be synergised by SPCSV in sweetpotato, both in terms of their titre according to DAS-ELISA and DNA hybridization and the development of leaf curling only in SPCSV co-infected plants. It is unusual for viral synergies that both SPLCV and SPCV are DNA viruses whilst the synergising virus, SPCSV [and most viruses previously reported to be synergised by SPCSV], is an RNA virus.

A main difference in the field between $SPCSV_{mld}$ and $SPCSV_{typ}$ is that $SPCSV_{mld}$ never appears to be co-infected with SPFMV. However, graft inoculating $SPCSV_{mld}$ infected cv Kampala white and other cvs with SPFMV led to infection with SPFMV and mild symptoms of SPVD. Consistent with these latter results, sequencing both its p22 and RNase 3 genes failed to reveal differences outside the reported range of variation of these genes for $SPCSV_{typ}$. The lack of co-infection of $SPCSV_{mld}$ and SPFMV in the field therefore remains unexplained. The coat protein (CP) and the minor CP of $SPCSV_{mld}$, both of which have recently been shown to provide RNA silencing in other closteroviruses, will be sequenced.

RNase3 and p22 genes from SPCSV had been transformed into sweetpotato and they are now being introduced into *Arabidopsis thaliana* for comparison and so that the extensive knowledge of the responses of this model plant can be exploited. We are also testing the hypothesis that SPVD is due to suppression of RNA silencing that normally excludes viruses from the meristem. The corollary, that recovery from SPVD is due to re-established meristem exclusion, is also being tested. These analyses involve *in situ* hybridisation to detect virus in and around the meristem of virus infected plants. Probes specific to SPFMV and SPCSV were made for *in situ* hybridizations. Preliminary results indicate SPFMV is not excluded from the meristem in three of the four tested cultivars New Kawogo (from Uganda), CIP 420020 and Beauregard (commercial variety) during active SPVD infection. Furthermore, cultivars tested New Kawogo, Beauregard and CIP 420020 and 420026 (from South America) are susceptible to SPVD infection caused by Ugandan SPFMV and SPCSV isolates. However, CIP 420020 seems to show a delayed onset of disease symptoms in comparison to the other three test cultivars with symptoms even appearing later and less severe than in New Kawogo, a Ugandan variety known to be resistant to SPVD.

3: Is resistance to SPCSV in sweetpotato an extreme of RS-based resistance or otherwise: The CIP clone DLP 3163 has been selected for and shown to possess extreme resistance to Peruvian SPCSV. However, graft inoculation with each of 14 isolates of SPCSV including $SPCSV_{mld}$ collected from different locations in Uganda infected it. Ugandan isolates of SPCSV seem unique in that most possess a p22 gene [confirmed for test isolates used] and previous work showed this to exhibit strong RNA silencing. Because DLP 3163 was identified as resistant through selection against Peruvian SPCSV [therefore lacking the p22 gene] it seemed likely that the p22 gene of Ugandan isolates is responsible for their virulence. This has now been disproved because Tanzanian isolates of SPCSV lacking the p22 gene have also now been shown to break the resistance of DLP 3163.

4: Are sources of resistance effective in the field and their deployment: DLP 3163 grown outside in the Ugandan Sweetpotato Program crossing blocks was severely affected by SPVD, consistent with laboratory tests above describing its susceptibility to graft inoculation to Ugandan and Tanzanian SPCSV, confirming it is unsuitable as parental material in East Africa.

NASPOT 11, known by farmers as Tomulabula, was selected by participatory plant breeding (PPB) from seeds of open-pollinated New Kawogo, an SPFMV- and SPCSV-resistant [but not immune] Ugandan variety in a previous DFID-funded project. BB/F004028/1 analysed and collated trial data which led to its release in 2010: broad disease resistance, including resistance to SPVD based on its inherited resistance to both SPFMV and SPCSV, was cited as one of its main attributes. This is the first ever national release of a sweetpotato variety bred by PPB anywhere in the world. Farmer interviews have shown that this variety satisfies the broad range of farmer-required attributes. It has already been disseminated widely in Luwero district; growers report marked improvements in their livelihoods, some even buying building plots, largely as a result of the creation of a new market for them - selling planting material to farmers and local NGOs.

A long term aim of the project is to develop an easier way than extensive yield trials by which breeders can identify sweetpotato genotypes likely to resist SPFMV infection. Enzyme-linked immuno-sorbent assays (ELISA) would provide a practical method of comparing titres and monoclonal antibody [MAb] -based ELISA have been shown elsewhere to detect SPFMV when not synergised by SPCSV. These have now been obtained and will be tested against a range of infected germplasm.

Setbacks: A recurring problem affecting the project has been difficulties in obtaining visas for developing country partners [despite stressing in applications that the project is UK DFID funded]. Dr Owor's arrival was delayed some 5mths; we have repeatedly failed for no satisfactory reason [but are still trying] to get a visa for Dr Cuellar to spend a few months with her at Cambridge. This has resulted in delayed research activities and reduced interaction between the UK-based and CIP-based researchers, with adverse effects on group dynamics. Although a group decision has not been finalised, a request for a no-cost extension is likely to be the main remedial plan.
The **Next Generation Sequencing** course funded by the SARID Capacity Building fund was ~6x oversubscribed and only Dr Mukasa was accepted. Wellcome Trust has been asked to advise us when further related courses are available for Dr Owor, Dr Cuellar and Peter Wasswa.

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. Polymerase chain reaction (PCR) assay
2. Reverse transcription and viral sequencing
3. Deep sequencing [Using siRNA particles and high performance computers to compute viral sequences]
4. Virus sequence databases and programmes designed for their statistical comparison
5. Virus specific indicator plants

Research resources available to developing country scientists during the reporting period:
Although equipment for PCR is available at both Makerere and NaCCRI, sweetpotato researchers including Peter Wasswa have experienced major problems in accessing consumables. Peter's return to Makerere has been linked with efforts ensuring the latter are available, particularly specific diagnostics, e.g., primer pairs. Dr Mukasa has attended UK-based courses on Deep Sequencing and computer analysis of nucleic acid sequences. Funds to purchase a high performance computer for Makerere University have been obtained through a SARID Capacity Building grant.

Resources for plate ELISA to work for SPFMV detection are being arranged for Makerere and NaCCRI.

Agricultural technologies of relevance to the poor (the practical application of research to develop new tools, skills and ways of working)

Participatory plant breeding (PPB) is a particularly appropriate tool for developing new crop varieties for marginal agro-ecologies and for poorer farmers. Fifty three varietal characteristics had previously been identified as particularly important for sweetpotato by farmers in Uganda. Data was collected with mainly women farmers [who are the majority of sweetpotato farmers] belonging to a farmer group, Tusitukire wamu Kabulanaka Farmers' Association (TUKAFA) and neighbouring farmers in Luwero District so as to examine how a clone selected by PPB during a previous DFID-funded project, the main local variety and the main conventionally-bred variety fitted these farmer criteria. Its analysis has shown that the PPB-bred variety fitted the broad range of farmer criteria better than either of the other varieties. This PPB bred clone, known by farmers as Tomulabula, was released as NASPOT 11 in 2010 by the Ugandan Sweetpotato Programme and 2 papers, one based mainly on on-farm and on-station trials and one on farmer responses in interviews, have been submitted to international journals. In filmed interviews, farmers, again mainly women, explain how the new variety has improved their lives, additional sales of planting material and higher prices for the roots paying school fees, clothes and even building land.

New scientific collaborations involving developing country partner(s) (collaborations consisting of at least one day per week)
The CIP-led 'Sweetpotato Action for Security and Health in Africa (SASHA)' project was funded by the Bill & Melinda Gates Foundation in 2009. It includes an East African sweetpotato seed systems research component which funds Dr Barker directly together with Dr Gibson, a Ugandan PhD student at Makerere University supervised by Dr Mukasa and capacity building at NaCCRI for the Ugandan Sweetpotato program. As well as enabling Dr Gibson to spend more time in East Africa with project partners, valuable additional crop samples have been collected and the Ugandan student's research builds on the ability of RNA-silencing based resistance in local Ugandan varieties to eliminate virus infection. A concept note for the wider promotion and dissemination of NASPOT 11 has been prepared for a Ugandan NGO to present to local funding sources. This may be BUCADEF which was funded by DFID's Crop Protection Programme to disseminate previous new sweetpotato varieties in Uganda.

Publications - production of research information (e.g. journal papers, book, book chapter, conference paper, policy brief, in-house publication[1], in-house published products[2]

List details (titles etc.) of publications during reporting period. Indicate whether papers/publications have been peer-reviewed externally and/or are open access. Specify whether lead author is a developing country researcher.

Cuellar, J. W. (2009) Molecular studies on the Sweetpotato virus disease (SPVD). 15th Triennial Symposium of the International Society for Tropical Root Crops. 2-6 November 2009. Oral presentation

Mwanga, R.O.M., Kigozi, B., Namakula, J., Mpembe, I., Niringiye, C., Tumwegamire, S., Gibson R. W., Yencho, C. 2010. Submission to the Variety Release Committee for release of sweetpotato varieties 2009. National Agricultural Research Organization (NARO). 41pp

Wasswa, P., Otto, B., Gibson, R. W., Maruthi, M. N., Mukasa, S. B. 2010. First identification of Sweet potato leaf curl virus in Uganda reveals its wide occurrence in the country. Plant Pathology [submitted]

Gibson, R. W., Mpembe, I., Mwanga, R. O. M. 2010. Benefits of participatory plant breeding (PPB) as exemplified by the first-ever officially released PPB-bred sweet potato cultivar, NASPOT 11. Economic Botany [submitted]

Mwanga, R. O. M., Niringiye, C., Alajo, A., Kigozi, B., Namukula, J., Mpembe, I. Tumwegamire, S. Gibson, R. W., Yencho G.C. 2010. Release of 'NASPOT 11' in Uganda, the first ever sweetpotato variety bred by a participatory plant breeding approach. Hortscience [submitted]

Underlined lead authors are developing country researchers; Underlined journals are peer-reviewed externally.
Four more papers for submission to international peer reviewed journals are in preparation.

Infomedia – requests for research information (infomedia is the means used to share knowledge, e.g. newspaper, TV, radio, mobile phones, websites, magazines)

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

The release of Tomulabula/ NASPOT 11 has been announced on the NRI website http://www.nri.org/

Interviews with farmers growing Tomulabula have been filmed with the aim of developing a short film.

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)

Provide a summary of the nature, duration and progress of any training provided as part of this project. Include number of delegates/students per item.
Mr Peter Wasswa completed his training on PCR and QPCR and on virus sequencing at FERA and returned to Uganda in November 2009 to conduct his field research on the virus resistance of Ugandan sweetpotato cvs and on the epidemiology and characterisation of SPLCV and $SPCSV_{mld}$ at Makerere's Kabanyolo Field Station and at NaCCRI.

Capacity Building Grant
Dr Mukasa attended a course on Next Generation Sequencing at the Wellcome Trust Genome Campus at Hinxton, Cambridge 19 – 23 July, 2010.

[1] Including working papers, newsletter, internal series. Points d) to g) above are also applicable to in-house publications.

[2] Including toolkits in CD form; videos

Dr Mukasa and Dr Owor attended a course on Advanced Computer Analysis of Nucleic Acid Sequences at Plant Sciences Dept, Cambridge University on 27 & 27 July, 2010.
The Next Generation Sequencing course involves modern developments in sequencing in which large numbers of small fragments of DNA are simultaneously sequenced to study how siRNAs respond to virus infection and computer programmes seek overlaps to achieve 'one shot' full length sequencing rather than conventional sequential sequencing. The subsequent course at Cambridge focused on the computer analysis of the siRNAs and of plant virus sequences.