PROJECT DETAILS

Grant number

BB/F004028/1

Award holding organisation

Organisation	University of Greenwich	Research Organisation Reference:	
Division or Department	Natural Resources Institute, Agriculture, Health & Environment Group		

Title of research project

How resistant plant varieties avoid suppression of RNA silencing by viruses as exemplified by sweetpotato: Better food security through virus control

Project details

Total grant value (£)	£601,906.42		
Start date	August 11 2008	Original duration of grant (months)	36
End date	August 11 2011	Extension (as agreed with BBSRC Office)	Not applied for

Investigators

Role	Name	Organisation	Division or Department
Principal investigator	Dr RW Gibson	University of Greenwich [UoG]	Natural Resources Institute [NRI]
PhD student[1]	Mr P Wasswa	UoG /Makerere University [MU]	NRI/ Crop Sciences
Co-investigator	Prof D Baulcombe	University of Cambridge [UC]	Plant Sciences
Co-investigator	Dr S Mukasa	Makerere University	Crop Science
Postdoctoral fellow[2]	Dr B Owor	Makerere University	Crop Science
Co-investigator	Dr I Barker[3]	International Potato Center [CIP]	Integrated Crop Management
Co-investigator	Dr J Kreuze	International Potato Center [CIP]	Germplasm Enhancement & Crop Improvement
Postdoctoral fellow[4]	Dr W Cuellar	International Potato Center [CIP]	Integrated Crop Management
Co-investigator	Dr I Boonham	Food & Environment Research Agency [FERA] [5]	FERA International
Co-investigator	Dr R Mwanga/ Dr G Ssemakula[6]	National Crops Resources Research Institute [NaCRRI]	Sweet Potato Programme

[1] Mr Peter Wasswa, BSc; MSc (MU), a Ugandan, registered at UoG & supervised in Uganda by Dr Mukasa

[2] Dr Betty Owor, BSc; MSc (MU); PhD (University of Cape Town, South Africa), a Ugandan; recruited by MU December 2008 and based at UC, Dept of Plant Sciences.

[3] Dr Barker was transferred from Head Office in Lima to the CIP Regional Office in Nairobi in August 2009. Dr Kreuze remains in Lima; any –ve impacts on project activities in Lima are likely to be counterbalanced in Uganda.

[4] Dr Wilmer Cuellar, a Peruvian, did his PhD in Finland on sweet potato viruses; recruited by CIP November 2008.

[5] Was Central Sciences Laboratory [CSL] but now re-named FERA.

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[6] Dr Mwanga was appointed [June '09] as the CIP Sweetpotato Breeder in Uganda. As CIP is a partner in BB/F004028/1, he will remain part of the project team. Dr Ssemakula is expected to replace him as the Head of the Sweet Potato Programme at NaCRRI. They are currently working together to support project activities.

Objectives - the main objectives of the research in order of priority

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.

No revisions to the Objectives were required by the awarding committee and no subsequent changes have yet needed to be agreed with BBSRC Office

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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

Summary report – a summary of progress on the grant (up to 2 A4 pages)

Progress of collaboration. Collaboration has progressed well with good links established between partners aided by recruitment (see page1) and exchange of suitably qualified personnel from developing countries, e.g., Dr Cuellar did his PhD on sweet potato viruses. Dr Cuellar is based at CIP Lima but is to spend 3mths (Aug – Sept, '09) at Cambridge, using the advanced facilities there, developing the collaboration and exchanging ideas, especially with Dr Owor. Dr Owor, based now at Cambridge, may later reciprocally visit CIP. Mr Wasswa, a graduate (First Class) of Makerere University, started at NRI in February reviewing literature and gaining experience in detection of viruses using indicator plants and Polymerase Chain Reaction [PCR] assays; he is now at FERA gaining experience in the more sensitive Real Time PCR. He will return to Uganda later this year for 2yrs where he will be supervised locally by Dr Mukasa. The Annual Project meeting was at Cambridge University on May 14th & 15th 2009, hosted by Prof Baulcombe. All project members as well as Dr Gowda (an NRI virologist and Mr Wasswa's second supervisor) and Dr Monger, a FERA virologist working with Dr Boonham attended. Each team member presented their work; ideas were exchanged and future activities were co-ordinated between team members. Dr Gibson has visited all team members and institutions to co-ordinate activities during the year, several visits in particular being made to Makerere University and NaCCRI, flights funded mainly by his other projects.

Progress of research: The project seeks to understand how *Sweet potato chlorotic stunt virus* [SPCSV] breaks resistance to/synergises *Sweet potato feathery mottle virus* [SPFMV] in sweetpotato and other viruses infecting sweetpotato more rarely in Africa, and the normal operation of this resistance in the absence of SPCSV. SPCSV was known to synergise SPFMV by blocking sweetpotato's gene silencing-based virus resistance: a greater understanding of this should 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:

- **Theme 1: Biological characteristics of sweetpotato/virus interactions, achieving a broad overview:** Brainstorming at the annual meeting identified a core combination of viruses: SPFMV, *Sweet potato leaf curl virus* [SPLCV] and SPCSV; and two cultivars differing in their resistances: New Kawogo [resistant to SPFMV and SPCSV but susceptible to SPLCV] and Beauregard [expected to be susceptible to all three] – there are no known sources of resistance to SPLCV. For different combinations of these viruses and varieties, virus titres, symptoms and the frequencies under different conditions of infected plants to recover [become asymptomatic] and revert [become virus-free] will be determined (Mr Wasswa's PhD research). Real time PCR allows sensitive and quantitative virus detection: preliminary results suggest that the titre of SPFMV is less in New Kawogo than in Beauregard. This core combination will be extended in Uganda using Ugandan and exotic landraces used in the NaCRRI breeding programme and with a wider range of resistances.

  The addition of SPLCV to the list of core viruses to be studied derives from our first ever detection of it in Uganda [It has been reported previously in sub-Saharan Africa only in Kenya in 2006]. Field samples indicate it is widespread and common, especially in New Kawogo, and, in studies outside Africa, it reduced yields appreciably yet was mainly symptomless. The Ugandan isolate similarly lacked specific symptoms in New Kawogo yet infected plants appeared less vigorous. Co-infection with SPCSV [and SPFMV] did not result in more severe symptoms, suggesting that, SPLCV, a DNA geminate virus, may be the first virus identified not

synergised in dual infection with SPCSV. The apparent susceptibility of New Kawogo to SPLCV also marks SPLCV as different from SPFMV and most other sweetpotato-infecting viruses. A Ugandan isolate of SPLCV has been partially sequenced, giving only an 89% maximum similarity to isolates from Asia**.** A South American isolate has been completely sequenced. Complete genome nucleotide comparison indicates SPLCV reported in USA as the closest related sequence (96% identity).

Two other DNA viruses infecting sweetpotato have been cloned and sequenced at CIP. For this a protocol using phi29 from sweetpotato samples has been developed. Phylogenetic analysis using Reverse Transcriptase (RT) regions group these viruses into the *Cavemovirus* genus (Fam. *Caulimoviridae*). Names suggested for these viruses are: "Sweetpotato cavemovirus A" (replacing sweet potato caulimovirus) and "Sweetpotato cavemovirus B" (replacing C-9 virus). Titres of Sweetpotato cavemovirus A increased in RNase3-transgenic sweetpotato as determined by NCM-ELISA along with symptom severity as compared with non-transgenic single infected sweetpotato. A similar increase in symptom severity has been observed in RNase3-transgenic plants infected with "Sweetpotato cavemovirus B", suggesting DNA spherical viruses are, like RNA flexuous rod potyviruses such as SPFMV, also synergised by RNase3 of SPCSV. An antisera against the recombinant coat protein of "Sweetpotato cavemovirus B" for NCM-ELISA tests is being prepared to confirm this.

- **Theme 2: Increased knowledge of RS-based resistance in sweetpotato controlling SPFMV:** Subsequent to the project's submission but prior to its start, team members at CIP transformed sweetpotato with different combinations of SPCSV's p22 and RNAse3 genes, the genes identified [see **Case for Support**] as likely to be responsible for blocking the sweetpotato's gene silencing-based virus resistance and so synergising SPFMV. Infection of transformed plants with SPFMV demonstrated that RNAse3 is mainly responsible for its synergy, a result confirmed by the identification of natural isolates of SPCSV lacking the p22 gene but still able to synergise SPFMV. Consequently, a recombinant SPCSV's RNAse3 protein has been purified using *Escherichia coli* for expression and affinity chromatography using histidine tags for purification. This purified recombinant RNase3 protein is being used at FERA to develop monoclonal and at CIP to develop polyclonal antibodies, one aim of which is to pull down RNAse3 and associated plant-derived molecules in SPCSV-infected sweetpotato. The mechanism by which RNase3 suppresses RNA silencing depends on its endonuclease activity; the characterization of its role in RNA silencing and plant defence will be extended in the collaboration with the UC Plant Science Dept.

  The project seeks to understand both how SPCSV blocks sweetpotato's gene silencing-based virus resistance and how the resistance operates under normal circumstances, i.e., when it is not blocked. A common feature of silencing-based virus resistance in plants is the exclusion of virus to a greater or lesser extent from the apical meristem. Sectioning of SPFMV-infected plants and *in situ* hybridisation, immuno-localization and laser capture microdissection are therefore being used at Cambridge to confirm if meristem exclusion has a role in SPFMV resistance. These experiments will provide an insight into virus localization in SPFMV susceptible and resistant varieties and provide virus accumulation profiles over time in single infections of SPFMV and SPCSV.
- **Theme 3: Is resistance to SPCSV in sweetpotato an extreme of RS-based resistance or otherwise:** Resistance to SPCSV occurs independent of resistance to SPFMV, e.g., DLP 3163; only a small percentage of progeny are resistant consistent with recessive resistance requiring multiple copies in this hexaploid crop.
- **Theme 4: Are sources of resistance effective in the field and their deployment.** NaCCRI has developed field data to support the release in Uganda of an SPVD resistant, high-yielding and otherwise superior sib of New Kawogo, NK1081L, selected by farmer participatory selection in previous DFID-supported research. That New Kawogo is resistant to SPFMV and most other viruses in the field has been supported by the easy identification of material free of SPFMV from field material. In this process, SPLCV was first identified in Uganda. Using PCR [which currently gives only a 50% detection rate] ~15% of plants from 5 fields were found to be infected, all fields having positive samples. Contrastingly, field samples of two other popular varieties, NASPOT 1 and Dimbuka, and also NK1081L, were negative although all could be infected experimentally. Marker Assisted Selection [MAS] is being done amongst progeny of a cross with the highly SPCSV-resistant DLP 3163 in Peru by CIP counterpart core research. That SPFMV-resistant New Kawogo appears to have a lower SPFMV titre than susceptible Beauregard suggests titre may be a useful selection criterion. Dried samples of SPFMV did not deteriorate over a 29 day period at FERA allowing samples obtained overseas, i.e., by NaCRRI Sweet Potato Programme, to be sent to UK for analysis.

Some logistical changes. Locational/job changes of Investigators are detailed on Page 1. The UoG will additionally fund a postdoctoral fellowship in 2009/10 from its core budget which will provide additional support to project activities. The delayed arrival of Dr Owor at Cambridge University (May 2008) made it inappropriate for Dr Mukasa to spend 4mths at Cambridge in Yr 1 and Dr Cuellar is to spend 3 months there instead.

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

Addition to my 2008/9 report:
Please note that Section 3 Outputs were not required in the format requested for the original 2008/9 report. Section 3 was requested and provided by/to BBSRC in July 2010, some 10mths later, based on the new reporting format. Though I have tried to restrict this addition to outputs delivered as of August 11, 2009 [project anniversary], I have occasionally taken the opportunity to indicate how these subsequently developed.

SECTION 3: OUTPUTS

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

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

The list above of research products in use by project developing country scientists during the reporting period is only of the **methods** being used. These methods automatically require access to a long list of equipment, e.g., thermocyclers for PCR, tools, e.g., statistical analytical packages, and consumables including both general laboratory chemicals as well as advanced molecules, e.g., primer pairs.

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 considered to be 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. Research 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 has examined 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. Analysis of this data in 2009/10 showed that the PPB-bred variety does indeed fit the broad range of farmer criteria better than either of the other varieties. Based on data from on-farm and on-station trials, this PPB bred clone, known by farmers as Tomulabula, was released as NASPOT 11 in 2010 by the Ugandan Sweetpotato Programme and 2 papers submitted to international journals.

New scientific collaborations involving developing country partner(s) (collaborations consisting of at least one day per week)

Please specify whether partnerships are led by the developing country partner and the nature of the resources (e.g. cash funds, staff, equipment) leveraged by the partnership. Plans for new collaborations not currently in operation may also be included here, but should be identified as such.

Developing country-led collaborations
Dr Wilmer Cuellar has provided supervision for four Masters students at the Peruvian National Agrarian University, La Molina, Peru.

Dr Gibson & Dr Barker have contributed to the development of the East African sweetpotato seed systems research component of a project entitled 'Sweetpotato Action for Security and Health in Africa (SASHA)' submitted by CIP to the Bill & Melinda Gates Foundation. This proposal includes funding strengthening national programmes and for a Ugandan PhD student This project was funded in late 2009. As well as enabling Dr Gibson to spend more time in East Africa in direct contact with project partners, it has enabled the project to collect valuable additional crop samples and a full time Ugandan student has just [May 2010] been recruited for PhD research building on the ability of RNA-silencing based resistance in local Ugandan varieties to eliminate virus infection.

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]

Accepted oral presentation: Cuellar, J. Wilmer (2009) Molecular studies on the Sweetpotato virus disease (SPVD). 15th Triennial Symposium of the International Society for Tropical Root Crops. 2-6 November 2009.
Dr Cuellar is a Peruvian national

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.

This report involves Year 1 activities and it was premature to announce any results.

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.

Dr Wilmer Cuellar has provided supervision for four Masters students at the Peruvian National Agrarian University, La Molina, Peru. He in turn has learnt New Generation Sequencing, otherwise known as deep sequencing.

Peter Wasswa, a Ugandan student at Makerere University, has spent 8mths in UK, 4mths at the Natural Resources Institute and 4 Mths at the Food & Environment Research Institute. As well as being trained generally in good laboratory practices, he received training in advanced virus identification and characterisation, including both gel electrophoresis and quantitative [real time] polymerase chain reaction [PCR] assays, designing primers and optimising assay conditions, and sequencing viral genomes.

[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