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

Case for Support: Part 1: *Track records, collaborations, facilities & expertise*:

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Dr Richard Gibson, project leader, has been involved in several sweetpotato research projects with scientists from developing and developed countries. In 1994, he was based in Uganda at the National Crops Resources Research Institute (NaCRRI) (formerly Namulonge Agricultural and Animal Production Research Institute) as part of a 3 year International Potato Center- (CIP) led DFID Holdback project (R5878) on control of sweetpotato viruses. The collaboration led to the identification of *Sweet potato chlorotic stunt virus* (SPCSV) in East Africa and its synergy of *Sweet potato feathery mottle virus* (SPFMV) to induce the severe sweet potato virus disease (SPVD) (Gibson *et al.*, 1998), the main disease of the crop. It also identified that most farmers' planting material is virus-free, yielding similarly to certified material (Gibson *et al.*, 1997). Collaboration with the Faculty of Agriculture Makerere University (FAMU) through an MSc student identified that SPFMV-infected plants of a virus-resistant landrace, New Kawogo, produce some SPFMV-free cuttings (Aritua *et al.*, 1998). This reversion provides a mechanism whereby farmers' planting material remains substantially free of virus. DFID Crop Protection Programme-funded projects (1996-2005: total value approx £930,000) on the epidemiology and control of SPVD in Uganda and Tanzania developed a range of farmer-validated strategies for controlling virus diseases. Dr Gibson also led an EU INCO-DEV project (2000–'03; value approx £650,000), involving FAMU but also Kenyan, S African, Swedish and German partners on the 'Identification and characterisation of sweet potato viruses in East and South Africa, and assessment of host plant resistance for sustainable production'.

Dr Robert Mwanga, the Ugandan Sweetpotato Program (USP) head/breeder at NaCRRI, with additional funding from McKnight Foundation, has made breeding virus resistant varieties a focus. When based at CIP headquarters in Lima as part of his PhD studies, he identified single genes for resistance to SPFMV and to SPCSV (Mwanga *et al.*, 2003) using markers to locate genomic positions. His very high yielding and SPVD-resistant variety NASPOT 1 has in particular been adopted widely in Uganda, an important achievement in a country where 20% of the country is undernourished (FAOSTAT). This variety is also being adopted in Tanzania. The partnership between NRI, USP and CIP has developed the first participatory breeding programme of sweetpotato (Gibson *et al.*, 2007), rapidly selecting clones which are already being adopted by farmers and trialled by USP, CIP and the Tanzanian Root Crops Programme; these activities are being extended further through the African component of Phase 3 of the DFID-funded Tropical Whitefly IPM Project.

Dr Jan Kreuze's PhD included sequencing SPCSV, determining its expression strategy and demonstrating SPCSV-encoded p22 and Rnase3 proteins are suppressors of RNA silencing (RS) (Kreuze *et al.*, 2002; 2005). It is hypothesized that the RS suppression mediated by these proteins inactivates resistance to SPFMV (and other viruses) in sweetpotato, freeing SPFMV from RS-based resistance to multiply uncontrollably and thus cause SPVD. Dr Kreuze also participated in the EU INCO-DEV project headed by Dr Gibson. Dr Kreuze has now joined the Germplasm Enhancement and Crop Improvement Division at CIP's headquarters in Lima. In a current collaboration between CIP and Prof Valkonen at the University of Helsinki, transgenic plants expressing both proteins have been obtained and will be tested to confirm this hypothesis.

Previous transgenic sweetpotatoes expressing RS constructs targeting SPCSV and SPFMV were found to be not resistant, despite the presence of short interfering (si) RNA in these plants indicating that RS was induced.

Dr Ian Barker was recently recruited from Central Science Laboratories (CSL) to lead the virology group at CIP. He has particular expertise in virus detection and his group members have identified diverse forms of resistance to SPCSV, SPFMV and the complex disease, SPVD.

Dr Settumba Mukasa (FAMU) conducted his PhD on genetic variability and in-plant interactions of the three important sweetpotato viruses in East Africa funded by BIOEARN, identifying that, like the potyvirus SPFMV, the ipomovirus *Sweet potato mild mottle virus* (SPMMV) is also synergised by SPCSV (Mukasa *et al*., 2006). He was also supervised by Prof Valkonen, then in Sweden, overlapping with Dr Kreuze. Dr Mukasa returned to FAMU to head sweet potato activities there including supervision of RUFORUM (Rockefeller) and BIOEARN (Sida/SAREC)-funded MSc and PhD students, their research including recovery from SPMMV and selection of superior virus-resistant germplasm.

Prof David Baulcombe's group at Sainsbury Laboratory (SL) is at the forefront of world research on how RS is a fundamental eukaryotic RNA surveillance system involving RNaseIII type enzymes and siRNA; this position is recognised by publication in pre-eminent journals including Nature, e.g., Baulcombe (2001; 2004). It has made major contributions to how siRNA unite with larger target RNA to guide an RNase to destroy the 'tagged' RNA, providing a main defence of plants against invading viruses and how they are also involved in long-distance signalling of RS. Susceptibility is associated with the infecting virus suppressing RS, another area in which the group has led (Hamilton & Baulcombe, 1999; Baulcombe, 2001; 2002).

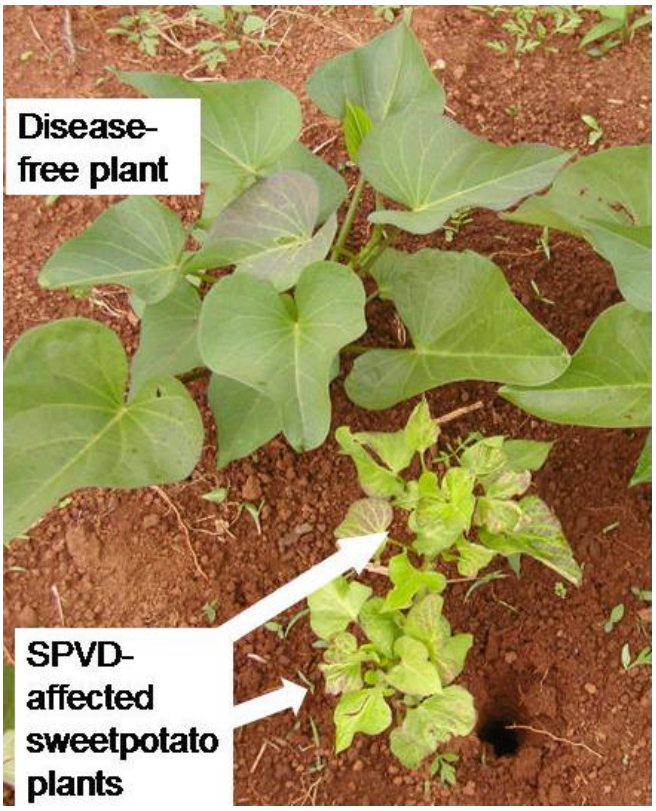
Dr Neil Boonham at the Central Science Laboratory (CSL) has particular expertise in very sensitive detection and quantification methods and runs a monoclonal antibody unit. CSL has broad responsibility for control of plant virus diseases of UK crops and its facilities and expertise provide an ideal background for initial virology training for the Ugandan PhD. CSL also has extensive virus collections including of criniviruses. Its responsibility for control of exotic viruses ensures it can provide legal and infrastructural facilities for importing and working with exotic sweetpotato germplasm and viruses.

The above demonstrates how our proposal includes a strong team of both developed and developing country scientists, relevant advanced expertise in virology, particularly RNA silencing combined with a broad background in sweetpotato virus management and a management team experienced in major international research projects. The past and continuing long-term collaboration amongst several project partners also demonstrates the high level of trust present. The partners are also involved in other major collaborations which will enable knowledge generated to be exploited effectively. This includes a US$6,000,000 Gates Foundation-funded programme in which CIP, NRI, USP and other national partners are deploying orange-fleshed (OF) sweetpotato in Africa to control vitamin A deficiency, particularly in children and nursing mothers. Our proposal addresses virus susceptibility, a common weakness of current OF varieties. CIP also has core funding to screen sweetpotato germplasm for attributes including resistance and to deploy identified genotypes. The SL is part of SIROCCO, a project focusing on studying RS in animals and plants. Funded under the Life Sciences, Genomics, and Biotechnology for Health Programme of the EU FP 6, it involves 18 advanced research institutions in ten countries. CSL leads projects on other criniviruses affecting European crops. MUK, the main source of degree-level agriculturalists in Uganda is closely linked to Swedish and Finnish universities through BIO-EARN to do advanced breeding and biotechnology with sweetpotato. NaCRRI leads Ugandan research on sweetpotato, is part of an East Africa-wide McKnight Foundation project on sweetpotato biotechnology and breeding and its Director chairs the Uganda's Steering Committee of the Africa-wide VITAA project.

Team members have extensive facilities to support proposed activities. NaCRRI has laboratory and tissue culture facilities, screenhouses, the East African regional crossing blocks for both OF and non-OF sweetpotato and an extensive research farm. FAMU has advanced virology and biotechnology laboratories as well as its own farm and laboratories. CIP with regional offices in Africa, has advanced biotechnology and virology laboratories in Lima and a worldwide germplasm for sweetpotato to exploit. CSL also has advanced and virology and biotechnology laboratories; SL has some of the most advanced biotechnology and virology laboratories in the UK but also provides an environment conducive to advanced plant molecular research as a result of the range of related ongoing activities in the Laboratory. NRI's, CIP's, FAMU's and NaCRRI's extensive activities in East Africa also brings institutional knowledge of a wide range of local background including informal partners (NGOs, farmer groups etc) that can be called upon. NRI, focusing on harnessing natural and human capital for the benefit of developing countries, also brings an office with staff dedicated to, and widely experienced in, handling the financial and other logistics involved in multi-national research projects, especially those overseas and involving developing countries.

Case for Support. Part 2: Proposed research (8 + 2 pages for research description because we are requesting 2 RAs + 1 diagrammatic workplan)

Introduction: Sweetpotato is the second most important root crop in East Africa; grown throughout sub-Saharan Africa particularly by poorer people, its production is increasing faster than any other staple food crop, up >40% since 1995. Viruses are a major constraint; the track records of project investigators and their partnerships, e.g., with Prof Valkonen's group in Helsinki provide background on sweetpotato-infecting viruses in Africa (reviews: Karyeija *et al.*, 2001; Gibson *et al.*, 2002; Tairo *et al.*, 2005). Most work has addressed sweet potato virus disease (SPVD) (see plate below), the most damaging disease of the crop in Africa and caused by co-infection of *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato chlorotic stunt virus* (SPCSV). SPCSV, which synergises SPFMV, drives spread of the disease and economic damage. Researcher- and farmer-validated control measures developed include varietal resistance, selection of unaffected planting material, roguing and isolation. Resistance is a preferred option by poorer farmers, being cost-free once initial cuttings have been obtained. Two recessive genes, *spcsv1* and *spfmv1*, providing resistance but not immunity to SPCSV and SPFMV respectively, have been identified in African sweetpotato (Mwanga *et al.*, 2002); new sources of extreme resistance have been identified by CIP in landraces from S. America.



The early focus on SPVD has taken the spotlight from the potentially more important problem of controlling SPFMV and other viruses causing mild or no symptoms in infected plants. Because farmers cannot recognise and select out affected plants (as they do for SPVD), infections can be uncontrollable in low-input cropping systems in vegetatively-propagated crops like sweetpotato. In commercial varieties, e.g., in S Africa, USA and China, the low yield of degenerated stocks has led to expensive cleaning and continual re-releases of virus-free stock. However, landraces grown without the support of virus-free schemes have yielded well for decades, e.g., in Uganda (Bashaasha *et al.*, 1995), some of these having such strong resistance to SPFMV to be able to eliminate it (Aritua *et al.*, 1998).

Previous research by project partners and collaborators has included sequencing SPFMV's monopartite and SPCSV's bipartite genomes, determining expression strategies and demonstrating p22 and RNase3 proteins on RNA1 of SPCSV, apparently uniquely encoded in plant viruses, are suppressors of RNA silencing (RS) (Kreuze *et al.*, 2002; 2005). RS, triggered by double stranded RNA (dsRNA), is a fundamental RNA surveillance system controlling gene expression in eukaryotes and is a key plant defence against systemic virus infection (Voinnet *et al.*, 1999; Waterhouse *et al.*, 2001). Short (21-26 nucleotide (nt)) interfering RNA (siRNA) (Hamilton & Baulcombe, 1999) is generated from target dsRNA by RNaseIII type enzymes, also known as Dicer (Baulcombe 2001) from their ability to cut dsRNA into many siRNA. These siRNA then serve as guides identifying homologous sections of dsRNA for cleavage and further degradation through an RNA-induced silencing complex (RISC). An RNA-dependent RNA polymerase is part of a system enabling siRNA propagation throughout a plant (Baulcombe, 2002; Schwach *et al.*, 2005). This degradation of dsRNA produced, e.g., during replication of invading RNA viruses provides the main viral defence in plants once infection has been initiated. Viruses have evolved means of suppressing RS; plant susceptibility is associated with a virus being able to suppress RS. Diverse viral genes have been identified with this role (Moissiard & Voinnet, 2004); the synergy of SPFMV by SPCSV to cause SPVD has been attributed to SPCSV's p22 and Rnase3 genes suppressing RS-based resistance (Kreuze *et al.*, 2005). This implies that SPFMV is normally constrained by RS in sweetpotato. The transient symptoms and recovery observed when SPFMV infects sweetpotato alone (Aritua *et al.*, 1998) are also hallmarks of RS-based resistance (Agral *et al.*, 2003;

Baulcombe, 2004). In the *Potyviridae*, RS suppression is a function of helper component proteinase (HC-Pro); although in sweetpotato (*I. batatas*), SPFMV's HC-Pro may be largely ineffective at suppressing RS, SPFMV HC-Pro allows *Potato virus X* (PVX) to spread in *Ipomoea nil* (Sonoda *et al.*, 2000).

SPFMV, the commonest virus of sweetpotato worldwide including Africa, occurs as several strains (common (C), russet crack (RC), ordinary (O) and East Africa (EA) strains) (Tairo *et al.*, 2005). All cause only mild or no symptoms in sweetpotato, virus titres are low, infection may be transient and all are synergised by SPCSV. Other potyviruses infecting sweetpotato include *Sweet potato mild speckling virus* (SPMSV), Sweet potato virus Y (SPVY = SPV2 = IVMV) and *Sweet potato virus G* (SPVG); there is also the ipomovirus *Sweet potato mild mottle virus* (SPMMV), the carlavirus *Sweet potato chlorotic fleck virus* (SPCFV) and the cucumovirus *Cucumber mosaic virus* (CMV). All appear to have similar properties when infecting sweetpotato, causing few or no symptoms, having low virus titres, infection may be transient and all are synergised by SPCSV (Karyeija *et al.*, 2000; Kokkinos & Clarke, 2006; Mukasa *et al.*, 2006; Untiveros *et al.*, 2007) suggesting the resistance of sweetpotato to them when they infect it alone is also RS-based. *Sweet potato leaf curl virus* (SPLCV; *Begomovirus; Geminiviridae*), Sweet potato leaf speckling virus (SPLSV; a possible polerovirus (*Luteoviridae*)), Sweet potato latent virus (SPLV) and Sweet potato caulimo-like virus (SPCaV) all affect sweetpotato mildly, but appear not to have been tested for synergy in co-infections with SPCSV. These commonalities, despite not being fully explored, seem remarkable and suggest some form of broad-spectrum RS-based resistance is present in sweetpotato, particularly in landraces in which the property is most evident. Resistance with a broad spectrum is of course essential in landraces of a vegetatively-propagated crop such as sweetpotato in which individual landrace clones may live for many decades and it is not restricted to this crop. Cassava mosaic disease- (CMD) resistant cassava, the most effective control found for the current CMD pandemic in East and Central Africa, is also RS-based (Chellappan *et al.*, 2004). As with sweetpotato, it resists all (albeit closely related) cassava mosaic viruses and infected plants recover to produce disease-free cuttings (Gibson & Otim-Nape, 1997).

Our proposal will investigate resistance in sweetpotato landraces, primarily against SPFMV but also other viruses including SPCSV, aiming to develop sweetpotato cultivars resistant to degeneration and SPVD whilst also increasing knowledge generally of RS-based resistance. SPCSV can infect plants in other species, genera and families than sweetpotato including the well-researched model virus host *Nicotiana benthamiana*; opportunity will also be taken to examine the range of hosts in which SPCSV's p22 and Rnase3 genes can cause synergy and thereby the scope of RS-based resistance in these other host plants too. Our proposal includes examining whether quantitative or qualitative characters of siRNA, the agents of RS-based resistance, can be used to directly identify resistant sweetpotato genotypes in breeding programmes and whether resistance is, e.g., a failure of SPFMV's HC-Pro to recognise and suppress sweetpotato's RS. Sweetpotato is ideal for the research: easy vegetative propagation provides genetically identical plants, germplasm collections in which resistant genotypes have already been identified provide host plant diversity, diverse viruses infecting the crop provide further scope. Virus resistance is a prerequisite for the crop in low input farming systems and our proposal includes partners in Africa with strengths ranging from well-equipped biotechnology facilities to facilities and experience in participatory breeding with sweetpotato.

The Planned Research Programme has four main themes:

Theme 1. Biological characteristics of sweetpotato/virus interactions, achieving a broad overview: As detailed above, interactions between sweetpotato and diverse 'mild' viruses infecting it have several apparently common factors suggesting the presence of broad-spectrum, RS-based resistance against them, particularly in landraces. The commonalities observed have not been tested methodically and, e.g., natural/human selection could have pyramided specific resistances against viruses occurring in the same region. Although members of the *Potyviridae*, a carlavirus and a cucumovirus are synergised in co-infections with SPCSV, species in other families remain untested. It is unknown whether SPCSV synergises other viruses in non-sweetpotato hosts. This set of activities tests the limits of these attributes whilst coincidentally collecting biological information valuable for control strategies. Project partners have access to all virus species infecting sweetpotato and to landraces from the Americas and Africa with high or extreme resistance to SPFMV when inoculated alone, e.g., from East Africa, New Kawogo and other landraces resistant to SPFMV and SPVD: from the Americas, **420020:** extreme resistance to SPFMV, not resistant to SPCSV or SPVD; **RCB 3014H & RCB3017H** – infectible by both SPCSV and SPFMV but SPVD-resistant.

Activities 1: Plants of SPFMV-resistant and susceptible sweetpotato from Africa (Af) and the Americas (Am) will be graft inoculated with single isolates of viruses which will include SPFMV strains EA (Af & Am), RC (Am) and C (Am) strains, SPMMV (Af), SPMSV (Am), SPCFV (Af), SPVG (Af & Am) and SPVY (Af & Am). Other hosts may be inoculated by sap. When necessary, trials will be conducted at CSL where advanced quarantine facilities located in UK where sweetpotato is not normally cultivated. Advanced diagnostic methods such as real time PCR will record changes in virus titres with time. Similar trials will test a wider range of viruses in co-infection with SPCSV and in non-sweetpotato plant species.

Objective 1.1: Determine the range of viruses to which SPFMV-resistant landraces are resistant, including whether ones from the Americas resist infection by African strains/ other viral species and *vice-versa* (completed Yr 1).

Objective 1.2: Test whether other sweetpotato-infecting viruses, especially non-potyviruses, including SPLCV, SPLSV, SPLV and SPCaV are also synergised by SPCSV (completed Yr 1).

Objective 1.3: Test whether co-infection with SPCSV enables normally non-sweetpotato-infecting viruses such as PVX to synergistically infect sweetpotato [CMV apparently can] (completed Yr 1).

Objective 1.4: Test whether SPCSV can synergise infection of other viruses [and, if so, what range of viruses] when infecting non-sweetpotato host plants including *N. benthamiana* (completed Yr 2).

Expected outcomes will include determining:

- The range of viruses resisted by SPFMV-resistant sweetpotato.
- Whether symptoms of infection for viruses other than SPFMV are typical of those associated with RS-based resistance (mild, transient symptoms; an early virus titre peak followed by decline etc).
- The range of viruses synergised by SPCSV in sweetpotato and thereby the range of viruses against which its RS-based resistance provides control in the absence of SPCSV to suppress it.
- Whether SPCSV can synergise viruses in other host plants and, if so, in which host plants and for which viruses, again.

These outcomes are expected to inform on the range of circumstances in which RS-based resistance can provide virus control in sweetpotato, providing clues as to the mechanism(s) of resistance. They will also indicate the practical value of these resistances and, from current knowledge of the distribution of different viruses, indicate their geographically useful range(s).

Theme 2. Increased knowledge of RS-based resistance in sweetpotato controlling SPFMV: SPCSV's suppression of RS-based resistance through its p22 and RNase3 genes is now a fairly-well understood system: both RNA1 and RNA2 of SPCSV have been sequenced and individual genes have been spliced into vectors for expression in experimental tobacco systems to induce suppression of RS-based resistance. SPFMV has been sequenced and there has been extensive research by others on the role of HC-Pro in the *Potyviridae* and of analogous genes in other virus families in suppressing RS. It seems likely that at least SPFMV HC-Pro, perhaps most of its homologues, and some analogous genes in other virus families are largely ineffective at suppressing RS in SPFMV-resistant sweetpotato whereas SPCSV's suppression of RS-based resistance through its p22 and RNase3 genes is highly effective. This theme seeks more specific details of RS-based resistance.

Activity 2: For SPFMV-resistant genotypes confirmed as potentially useful in Activity 1, symptoms, virus titres, timescale and other characteristics of infection will be determined for plants infected or not infected by the following viruses with and without SPCSV:

- SPFMV of EA, RC and C strains [the C-strain is only slowly synergised by SPCSV].
- An SPFMV clone expressing SPCSV encoded proteins [to confirm their involvement in synergism].
- An SPFMV clone tagged, e.g., by fluorescent GFP* to examine how SPFMV replicates and moves in the different genotypes [to determine at which stage of infection resistance is effective].

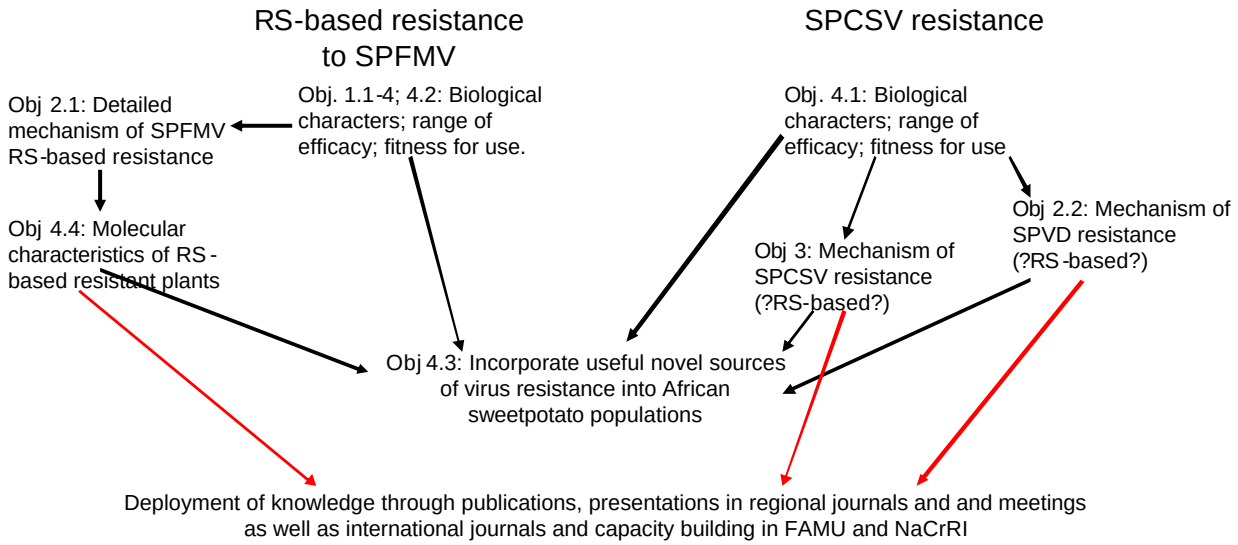
Other viruses will be included depending on the results obtained in Activity 1. SiRNA profiles will be compared using established gel electrophoretic methods for both total amounts and the presence or absence of particular sizes in infected and blank inoculated plants. Infection by SPFMV should induce specific siRNA; infection by SPCSV should suppress it. We also plan to test if specific plant molecules are co-purified with SPCSV's p22 and RNAse3 and SPFMV's HC-Pro using antibodies against these proteins. Most trials will involve the advanced facilities for analysing virus/host plant interactions at SL.

Objective 2.1: Identify the range of SPFMV strains and other viruses affected (completed Yr 1), molecular basis (completed Yr 3) and phenology (completed Yr 3) of natural host plant resistance to SPFMV.

Objective 2.2: Determine whether or not resistance to SPVD involves SPCSV being less able to suppress RS against SPFMV in some genotypes (completed Yr 2) and compare it with resistance in sweetpotato silenced by SPFMV transgenes (completed Yr 3).
Expected outcomes: Experiments are expected to provide detailed information on how sweetpotato resists SPFMV and other 'mild' viruses, similarities or otherwise of natural RS-based resistance with transgenic resistance and to identify molecular characteristics of resistant sweetpotato enabling them to be identified. We expect our research to confirm that SPCSV synergises SPFMV through suppressing RS-based resistance.

Theme 3. Is resistance to SPCSV in sweetpotato an extreme of RS-based resistance or otherwise: Resistance to SPCSV is the key to controlling SPVD. To achieve this, we already have identified: **DLP 3163** - extreme resistance to SPCSV, not resistant to SPFMV but SPVD resistant; **Transgenic sweetpotato*** - silenced against SPCSV and SPFMV yet not SPVD-resistant; **East African cultivars** - resistant to $SPCSV_{EA}$ from East Africa, SPVD-resistant; **West African cultivars** - resistant to $SPCSV_{WA}$ from West Africa, SPVD-resistant but susceptible to $SPCSV_{EA}$. We also have $SPCSV_{WA}$ from West Africa and with $SPCSV_{EA}$ from East Africa, different natural isolates of the latter being with and without a p22 gene.
Activities 3: We will challenge the above genotypes with $SPCSV_{WA}$ and with $SPCSV_{EA}$ with and without its p22 gene, measuring symptoms, virus titres, siRNA quantities and profiles and other attributes.
Objective 3: Determine whether natural resistance(s) to SPCSV and SPVD involves it being less able to suppress RS-based resistance or whether it involves some other form of resistance (completed Yr 2); compare natural resistance with genetically engineered resistance in sweetpotato silenced against SPFMV and SPCSV (completed Yr 3).
Expected outcomes: SPCSV is the key pathogen in the epidemiology of SPVD and also of many other viruses such as SPFMV because it is mainly when SPCSV co-infects that these viruses reach titres sufficient 1) to cause damage and 2) to be acquired by their vectors and spread to other plants. Information developed will determine whether sources of resistance to SPCSV above all have the same or different mechanisms, whether any is RS-based, whether they can be pyramided to provide stronger and more durable resistance and whether any are effective against both $SPCSV_{WA}$ and $SPCSV_{EA}$ strains.
* *Currently planned research with transgenically altered organisms is to be at CIP where such work is already ongoing and permissions and biosafety facilities are available. Specific licences giving permission for such experimental work at Sainsbury Laboratory will be sought if required.*

Flow Diagram of research



Theme 4. Are sources of resistance effective in the field and their deployment: Activities 1, 2 & 3 will have characterised virus resistances in different genotypes, confirming which are potentially useful in Africa.

Specific molecules, e.g., siRNAs, associated with resistance and methods for identifying such molecules should also have been achieved.

Activity 4: Apparently useful sources of resistance to African viruses will be tested for their field responses in Uganda. Materials that initial field tests have confirmed are useful will be incorporated into crossing blocks at NaCRRI, providing progenies to check for association of resistance and specific molecular markers, and enabling early incorporation into breeding programmes. Methods for identifying molecular indicators of resistance will also be transferred and tested in Uganda, linking to breeding programmes. Farmer involvement in the latter will be emphasised by participatory methods already deployed for sweetpotato in Uganda(Gibson *et al.*, 2007).

Objective 4.1: Confirm efficacy of DLP 3163 and other potentially useful germplasm from S. America for field resistance to African SPCSV isolates (completed Yr 1).

Objective 4.2: Analyse the significance of reversion, a hallmark of RS-based resistance, in maintaining farmers' planting material free of SPFMV and other mild viruses (completed Yr 2).

Objective 4.3: Incorporate novel sources of virus resistance into African sweetpotato breeding programmes using participatory breeding approaches (continuously during lifetime of project and continued afterwards).

Objective 4.4: Test whether pre-screening for siRNA or other molecules can identify virus resistant seedlings (completed Yr 3).

Expected outcomes: These activities aim to rapidly incorporate novel sources of resistance and novel means of identifying resistant genotypes into African breeding programmes. USP crossing blocks are used by CIP to provide seed for the entire East African region and clones released by USP are available to other national programmes through CIP's regional quarantine service in Nairobi enabling rapid regional deployment.

Scientific feasibility of project:

1. The project builds on extensive molecular knowledge of SPFMV and SPCSV [including both fully sequenced and some understanding of how SPCSV overcomes RS-based resistance in sweetpotato] and some 15 yrs of fundamental research on how plants control viruses through RS and how viruses combat RS.
2. The project team includes the UK's foremost research expertise and facilities on RS silencing of viruses (the Sainsbury Laboratory) and leading scientists experienced specifically in sweetpotato molecular virology, transformation and RS. Techniques for isolating components of RS against viruses, e.g., siRNA, DICER and RISC and of viruses against RS, e.g., HC-Pro are established. We have access to diverse sweetpotato a) virus and b) germplasm; virus resistant genotypes have already been identified. The team also includes East Africa's most active sweetpotato breeding program, providing germplasm not only for Uganda but also the region.
3. The team has considerable social capital, most team members have worked together closely in other research projects; the team leader has extensive experience in successfully leading international research teams including both developing and developed country scientists; NRI has extensive experience in their logistics.

Table 1. Main activities and locations of PhD student, FAMU RA and CIP RA during proposed studies.

PhD student	FAMU RA	CIP RA
Training in virology	Obj 2.1: Fundamental studies on the process of RS-based resistance in sweetpotato to SPFMV and other viruses. Identify key molecular features of RS-based resistance and of resistant genotypes	Obj 1.2, 1.3 & 1.4: Determine the range of viruses and host plants in which SPCSV can induce synergy.
Obj 1.1: Identify range of virus strains & species resisted by SPFMV-resistant sweetpotato		Obj 2.2: Determine whether RS-based resistance is involved in resistance to SPVD
Obj 4.1: Field efficacy of new sources of resistance to SPFMV, SPCS, SPVD		Obj 3: Understanding the mechanism of resistance to SPCSV
Obj 4.2: Field efficacy of reversion	Obj 4.4: Transfer identifying RS-based resistant sweetpotato to Ugandan breeding programme	
Write up thesis & papers		

Key: CSL | FAMU/NaCRRI [FAMU's field station is adjacent to NaCRRI] | SL | CIP

Structure and Management: The project has research bases and activities in three countries: UK (NRI, SL and CSL), Uganda (FAMU and NaCRRI) and Peru (CIP). Two full-time post-doctoral research assistants (RA) will be recruited, one by CIP and the other by FAMU. A PhD student will be registered at NRI with the

University of Greenwich and UK co-supervised by Dr Gibson and Dr Mukasa. A technician will support breeding activities at NaCRRI. Table 1 shows how activities will flow one into the other and how the RAs and PhD student will move to the most appropriate location. Dr Mukasa will also do research in UK on 2.1 to facilitate 4.4 in Uganda. Responsibilities will be divided in the following way:

Management: Dr Gibson is very experienced in the management of North/South international projects – see track record. NRI has a team of experienced project controllers plus contracts officer, dedicated computerised budget system and finance department to support him in the management of the project [covered by indirect costs]. They will arrange contracts setting out roles and responsibilities of each partner.

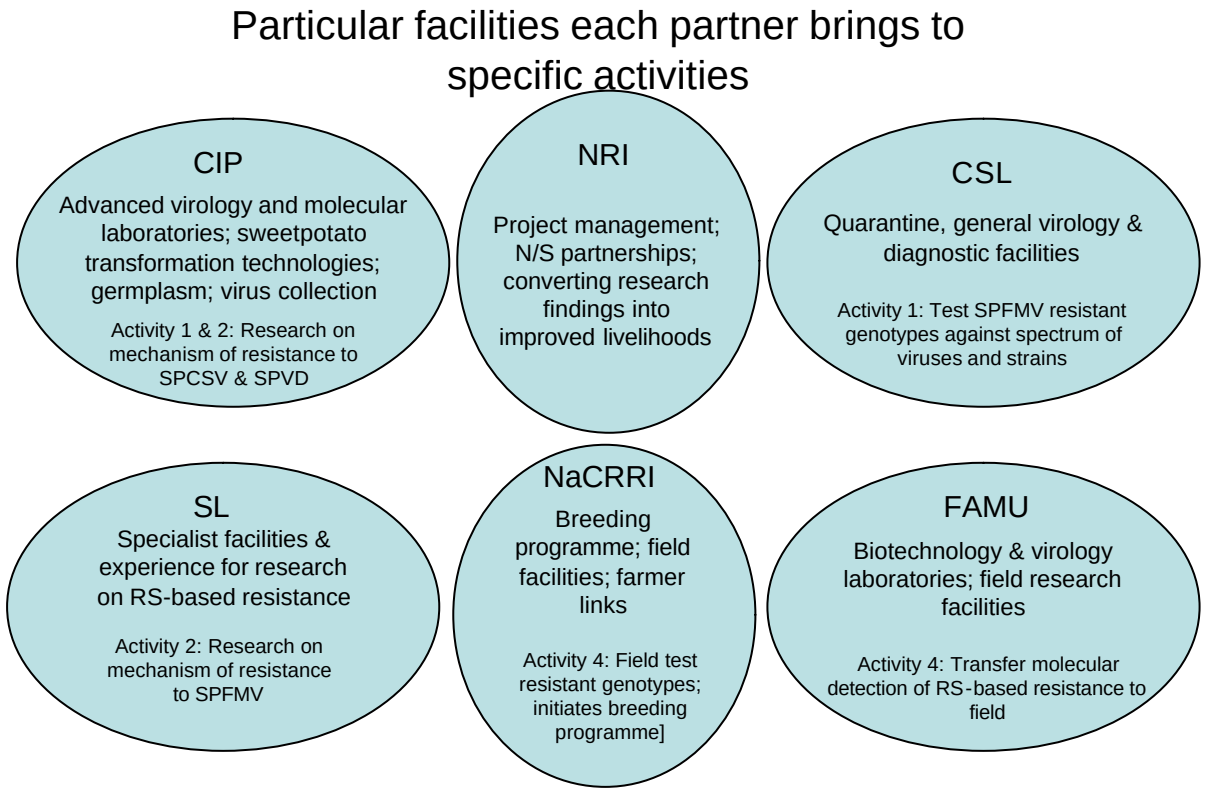
Research on broad characters of SPFMV-resistant sweetpotato: CSL will lead on activities 1.1 & 1.2, involving the PhD student and utilising its excellent containment and virology facilities and drawing on its and other partners' diverse virus collections.

Research on RS-based resistance to SPFMV: SL will lead on activity 2, drawing on its advanced facilities for research on RS and its experience in RS-based resistance in various experimental host plants.

Research on resistance to SPCSV: CIP will take the lead for activities 1.3, 1.4 & 3, drawing on its investigators' previous experience in research on SPCSV synergism of SPFMV and with sweetpotato transformation, diverse germplasm, excellent virology and molecular facilities etc.

Deployment of resistance: A technician will be recruited at NaCRRI and the PhD student will return to Uganda end of year 1 to support sweetpotato breeding. NRI has specialist experience in participatory breeding. Dr Mukasa will lead the virology and molecular work at FAMU, linking with SL and with the FAMU RA before s/he returns.

Exchange of technical knowledge amongst partners. The PhD student and RAs will move between advanced laboratories and to Uganda, carrying knowledge between locations (Table 1). Dr Mukasa will also spend time at SL, acquiring advanced skills for RS research. There will be team annual meetings occurring in UK, Africa and Peru as well as frequent interaction through email. Principal investigators will also make short exchange visits between UK, Peru (CIP) and Uganda. Dr Gibson will collate and circulate reports needed by BBSRC.



Transnational added value and capacity building: The two RAs and the PhD student will be developing country nationals. A potential candidate for the CIP RA is a Peruvian, Wilmer Chuquiyuri (CV also attached), now finalising his PhD on molecular aspects of sweetpotato viruses with Prof Valkonen at Helsinki University. The FAMU RA will be recruited within East Africa; again a potential candidate who will have completed her PhD studies in S Africa has been identified [A woman postdoc, although fortuitous, is potentially advantageous

for a project on sweetpotato, a 'woman's' crop in many parts of Africa]. The PhD student is expected to be a FAMU MSc graduate of Dr Mukasa. The FAMU RA based primarily in UK at SL will benefit from working in one of the UK's most advanced virology laboratories; the CIP RA may conduct limited research at CSL using its advanced containment facilities. The PhD student will receive virology training at CSL. Dr Mukasa will spend periods in UK linking with Dr Baulcombe and the FAMU RA whilst addressing Objective 2.1 and both will transfer knowledge of RS-based resistance back to Makerere (Objective 4.4) in the final year. This extensive involvement, exchanges and capacity building of developing country scientists provide **evidence of true cooperation within the collaboration** and will ensure knowledge moves seamlessly to them. Rapid exchange of germplasm will similarly be achieved. NRI, CIP, SL and CSL investigators will all visit Uganda, becoming more aware of regional needs and contributing to objectives 4.3 and 4.4

Scientific merit and Novelty of the proposal:

1. Studying RS-based resistance to virus infections in vegetatively propagated sweetpotato African and American landraces selected in low-input farming systems provides both novelty and merit because resistance is extreme and apparently broad-based. This contrasts with the low levels of resistance common in annual crops and the virus indicator plant species usually used in RS research, with the specificity of resistance from viral transgenes engineered to 'silenced' plants and with the highly specific resistances commonly used in breeding.
2. Our proposal tests for the first time whether aspects of siRNA or other specific agents of RS-based resistance can act as markers to identify resistant genotypes. Since RS-based resistance is a general plant mechanism to resist viral infections, success will be a major achievement both for crop improvement generally and in terms of fundamental knowledge of virus:plant interactions.
3. Our proposal combines for the first time a 'high-tec' selection procedure for identifying resistance with participatory breeding to achieve successful adoption. Being involved in plant variety selection can be very time and resource consuming for farmers, especially problematic for the poor and more vulnerable with limited land and no capacity to hire labour. Combining a high-tec method to ensure most genotypes in a population have the desired attributes with participatory selection by farmers would seem to have many advantages yet it is novel for the scientist component of participatory plant breeding teams to include molecular skills.
4. In sweetpotato, virus:host interaction studies have previously focused on understanding how SPCSV synergises SPFMV to induce SPVD. Our proposal is also novel and merits support because it instead targets the [more important] resistance which enables sweetpotato to avoid degeneration during long-term vegetative-propagation in low-input systems. Without this, sweetpotato and other vegetatively-propagated crops would be unable to sustain livelihoods of vulnerable people in Africa and other developing countries.

Relevance of project outcomes to development, how work will be taken forward and impact on poverty:

With an annual production of 11.5×10^6t in 2005 [http://faostat.fao.org/], sweetpotato is Africa's third most important root crop. Uganda, where it is the second most important root crop, has an annual production of about 2.6×10^6t, the greatest in Africa. It is an increasingly important source of food, food security (Bashaasha *et al.*, 1995), nutritional security and income (Scott *et al.*, 1999) for poorer farmers. Its high productivity, exceeding dietary energy produced/ha/day of maize by about 40% (Woolfe, 1992), is particularly important in countries like Uganda where 26% of children are underweight (http://earthtrends.wri.org/). Sweetpotato's indeterminate growth enables it to tolerate rains that, with climate change, are increasingly unpredictable; that it requires only light labour, yields something even on poor soil, requires little land since it can generate high Kcal/ha and can be piecemeal harvested over many months (and, in some environments, throughout the year) make it essential to women farmers as a source of daily family food (Bashaasha *et al.*, 1995). On the same basis, it is also being deployed by NGOs and governments to HIV AIDS affected families, displaced families, families in war-ravaged areas etc. Unlike most food crops in Africa (particularly maize), sweetpotato production is growing faster than the human population, up by nearly 40% since 1995 [http://faostat.fao.org/], almost doubling in Tanzania (FEWS data). This increase has occurred largely without government support and without market saturation, many of the countries being in chronic food deficit. However, sweetpotato is not just a crop for the "poorest of the poor"; sold into urban markets and made into diverse food products (Scott *et al.*, 2000), it also provides a way out of poverty (Scott *et al.*, 1999). As well as being a major carbohydrate source, the outstanding potential of sweetpotato is to combat vitamin A deficiency (VAD) through orange-fleshed (OF), high ß-carotene varieties - in SSA, 37% of preschool children (18.6 million) are affected by VAD (The Micronutrient Initiative, 2001; www.cipotato.org/vitaa/about_vitaa.htm VITAA project). Sweetpotato is also a major source of vitamin C, also B, E and folic acid, some minerals especially K and P, Zn, dietary fibre and

protein (Woolfe, 1992). CIP and NRI, collaborate in the HarvestPlus Programme addressing VAD through OF sweetpotato in Africa. This includes a US$6,000,000 Gates Foundation-funded programme in which CIP, NRI, USP and other national partners are collaborating to deploy orange-fleshed sweetpotato in Africa as part of an associated Challenge Programme. NaCRRI's Director also chairs the VITAA partnership in Uganda.

Viruses cause the main disease of sweetpotato in Africa (Geddes, 1990), virus susceptibility has been a particular weakness of exotic OF varieties and CIP models indicate that R&D on virus control alone could yield rates of return of up to 125% (total potential). Resistance, though often identified as appropriate for resource-poor farmers in developing countries because it is a low cost technology, is in practice much better than that because resistant new varieties generally also introduce additional benefits. The location of project activities within NaCRRI, the national and regional breeding centre for OF and non-OF sweetpotato, will facilitate the rapid development and deployment of virus-resistant OF and non-OF varieties. Virus resistance is already a top priority for CIP and NaCRRI and the sweetpotato breeding programme at NaCRRI has a successful track record of breeding the NASPOT series of virus-resistant varieties in Uganda (Mwanga *et al.*, 2003). Rapid adoption in Uganda, particularly NASPOT 1, has proved them both farmer- and market-preferred; this same team will be involved in incorporating novel resistances developed during project activities. Regional quarantine of planting materials in Nairobi, in which CIP is closely involved, enables easy regional exchange and provides another proven track by which new sweetpotato cultivars developed by project partners can be rapidly deployed regionally, particularly in the Great Lakes region where sweetpotato production in Africa is concentrated. Seed obtained from crossing blocks at NaCRRI is also distributed throughout sub-Saharan Africa by CIP networks. Inclusion of CIP and NaCRRI provides long-term breeding capacity. NaCRRI already holds a McKnight Foundation project [http://mcknight.ccrp.cornell.edu/projects/spu/uganda_sweetpotato.html] targeting 'Multiple resistance and high-yielding sweetpotato: Development of high-yielding, multiple-resistant sweetpotato germplasm' expected to be extended for a further 5 yrs. Both CIP and NaCRRI have linked with NRI to develop a participatory breeding approach for sweetpotato in which farmers have rapidly selected virus resistant clones. This specifically achieves early widespread adoption of new resistant varieties through their appropriateness to the local needs of farmers and the community and helps our work target the more vulnerable; our planned research will also develop further this successful approach.

Dissemination of knowledge: Vegetatively propagated crops such as sweetpotato and cassava, as well as dominating agricultural production in many African countries (FAOSTAT), are disproportionately important sources of food for poorer and more vulnerable people. RS-based resistance [rather than vector-based avoidance or resistance] is particularly important in vegetatively propagated crops because systemic infections are propagated in vegetative propagules unless RS-based resistance prevents it. There is currently little research on-going in Africa on mechanisms of virus resistance in crop plants including RS-based and its expansion will be supported by the direct involvement of developing country scientists, research assistants and PhD student (see in the research itself both in advanced research facilities in UK and in exploiting the outcomes using advanced laboratories available in local advanced research institutes. The location of the proposal within FAMU, where many agricultural researchers are trained for Uganda as well as East Africa, and NaCRRI, where most research on other vegetatively-propagated crops such as cassava and bananas in Uganda is also conducted, will facilitate this. The African Crop Science Society, publishing the *African Crop Science Journal*, is also based at FAMU. In addition to publication of research results in high impact international journals, partners will publish and present results in such Africa-based international journals and at African international and regional meetings. Another long-term outcome of this work is therefore the rapid spread of knowledge of RS-based resistance amongst African researchers including the initiation of sister research projects. A very early likely development is the directed selection of RS-based resistance to control cassava mosaic viruses.

Environmental impact: Virus resistance in itself is broadly neutral in environmental impact. Given that food production is essential, increased production of sweetpotato will reduce environmental impact since its greater productivity than other major staples allows less land to be cultivated. Its spreading habit also prevents soil erosion. However, perhaps its most important attribute is that its indeterminate growth makes it particularly robust in the face of climate change. Particularly severe environment damage is often caused by people during times of extreme stress, when they have no alternative but to utilise the few remaining natural resources. Increased production of sweetpotato prevents the occurrence of such desperate times by being able to survive and produce food following both flooding and short periods of intense drought [and being difficult to steal].

Engagement between academics and non-academics: The non-academics involved in this research are primarily African, specifically Ugandan farmers. Their knowledge and experience will be engaged indirectly by the focus of the project on landraces developed and used by farmers and directly by a participatory breeding approach (Gibson *et al.*, 2007). In this, farmer groups work collegially with researchers to select within segregating seedling populations grown by the farmers on their farms. This approach has been developed over the last several years by research in Uganda and Tanzania involving both Dr Gibson and Dr Mwanga. Farmers' varietal needs have been expressed and collated, the ability of the method and the capacity of farmers to select effectively has been validated by the rapid identification of resistant genotypes with high yields and other characters which are already resulting in their rapid adoption. The challenge this new project will address is to incorporate high-tec means of selecting resistance into a participatory breeding approach and including the orange flesh trait to provide the added benefit of alleviating vitamin A deficiencies.

Plate 1. Farmers discussing usefulness of different sweetpotato clones during participatory breeding. **Insert:** A root of one of the clones selected within just 3 yrs.

Arrangements for management of intellectual property (IP) & sharing of data

Research will be co-ordinated as team activities, with information and materials, e.g., germplasm with identified resistance passing freely between members. This will be ensured by long-term exchanges [e.g., Table 1 illustrates how RAs and PhD student move between locations] and by investigators making short-term visits including annual project team meetings in UK, Peru and Uganda. The main expected outputs from our publicly-funded project activities are knowledge of virus:host plant interactions, particularly of sources of resistance in sweetpotato and knowledge of their mechanism(s), scope, molecular identity and identification, and efficacy. Research findings will be presented in public fora, mainly by publication authored by all involved researchers in international refereed journals and as soon and as comprehensively as possible, but also by presentation in international and regional, particularly African, scientific meetings. IP is an issue with regard to co-authorships and all publications, formal or otherwise, in scientific journals or elsewhere, must pass by the project management team to verify that the authors do not infringe others' IP. The project management team will also certify the quality of papers to be published with reference to the project; feedback will be passed to the authors within 30 days of receipt of draft manuscripts. Sequence data will be submitted to EMBL database.

Project activities are likely to lead to the release of new sweetpotato varieties in East Africa; new sweetpotato varieties have never attracted levies there, new varieties being generated through public rather than private funds and the crop being subsequently propagated largely in the informal sector as a result of vegetative propagation ensuring varieties remain true to type. This circumstance seems unlikely to change in the near future so commercial aspects of new varieties are not expected to be an issue. We recognise, however, that there may be issues related to use of resistance from landraces and will abide by the Convention on Biological Diversity, and nationally recognized legal acts, e.g. Plant breeders rights, should they arise.

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

<table>
<tr><th>Objectives addressed</th><th colspan="4">Year 1</th><th colspan="4">Year 2</th><th colspan="4">Year 3</th></tr>
<tr><td>1.1: Determine the range of viruses to which SPFMV-resistant landraces are resistant.</td><td colspan="2">PhD student at CSL & NRI</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>1.2: Test whether other sweetpotato-infecting viruses, especially non-potyviruses are also synergised by SPCSV.</td><td colspan="4" rowspan="3">CIP research assistant with support from CIP virology group</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>1.3: Test whether co-infection with SPCSV enables normally non-sweetpotato-infecting viruses to synergistically infect sweetpotato.</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>1.4: Test whether SPCSV can synergise infection of other viruses when infecting non-sweetpotato host plants</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>2.1: Identify the range of SPFMV strains and other viruses affected, molecular basis and phenology of natural host plant resistance to SPFMV.</td><td colspan="8">FAMU research assistant with support from Sainsbury Laboratory</td><td></td><td></td><td></td><td></td></tr>
<tr><td>2.2: Determine whether or not resistance to SPVD involves SPCSV being less able to suppress RS against SPFMV.</td><td colspan="8" rowspan="2">CIP research assistant with support from CIP Virology group and Germplasm Enhancement and Crop Improvement group</td><td></td><td></td><td></td><td></td></tr>
<tr><td>3: Determine whether natural resistance(s) to SPCSV and SPVD involves it being less able to suppress RS-based resistance.</td><td colspan="4"></td></tr>
<tr><td>4.1: Confirm efficacy of DLP 3163 and other potentially useful germplasm from S. America for field resistance in Africa.</td><td></td><td></td><td></td><td colspan="4">PhD student at FAMU & NaCRRI</td><td></td><td></td><td colspan="2" rowspan="2">PhD student writing up at NRI</td></tr>
<tr><td>4.2: Analyse the significance of reversion in maintaining farmers' planting material free of viruses.</td><td></td><td></td><td></td><td></td><td></td><td colspan="4">PhD student at FAMU & NaCRRI</td><td colspan="2"></td></tr>
<tr><td>4.3: Incorporate novel sources of virus resistance into breeding programmes using participatory breeding approaches.</td><td colspan="12">Breeding activities co-ordinated within the Ugandan Sweetpotato Program at NaCRRI with support on the participatory approach from NRI</td></tr>
<tr><td>4.4: Test whether pre-screening for siRNA or other molecules can identify virus resistant seedlings.</td><td></td><td></td><td></td><td></td><td></td><td colspan="7">Dr Mukasa working with FAMU research assistant</td></tr>
</table>