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		
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Progress of work against project objectives

1. Biological characteristics of sweetpotato/virus interactions, achieving a broad overview.

Sequences of the coat protein region of isolates of West and East African strains of *Sweet potato chlorotic stunt virus* (SPCSV) from Africa and the Americas were similar. However, differences in the sequence of the hsp70 region enabled the identification of a third strain in central southern Africa named the South African strain. An isolate of SPCSV with negligible symptoms in the indicator plant *Ipomoea setosa*, purpling in sweetpotato and, unlike the wild type strain, seldom being co-infected in the field with *Sweet potato feathery mottle virus* (SPFMV), has been found commonly in a popular cultivar in Eastern Uganda. It still gave a 50% yield loss and experimental co-infection gave rise to only moderate SPFMV titres. Cross protection against the wild type was partial.

Detection of pararetroviruses had been difficult in sweetpotato in the past because they are symptomless, difficult to purify and no sequence was available. Complete genomes of two such cavemoviruses named *Sweet potato collusive virus* (SPCV) and *Sweet potato vein clearing virus* (SPVCV) have been obtained from Central America, Caribbean islands and East Africa. Primers for their detection by PCR have been tested and published, and their variability studied. Their genomic organisation resembles that of other *Caulimoviridae*; phylogenetic analysis shows they are most closely related to *Cassava vein mosaic virus* and *Tobacco vein clearing virus*, currently placing them in the related genera *Cavemovirus* and the newly formed *Solendovirus*. Their means of transmission was not identified; aphids did not transmit them. The presence of SPVCV has been shown for the first time in Africa [Uganda & Kenya]. A new carlavirus previously known as C-6 has been characterized. It has not yet been reported in Africa and is only 55% identical in amino acid sequence to *Sweet potato chlorotic fleck virus* (SPCFV), a carlavirus found in Africa. SPCV, SPVCV, the new pararetroviruses and C-6 are all

synergized by SPCSV; that of SPCV and SPVCV being the first synergistic interaction recorded between a DNA and an RNA virus.

A sweetpotato begomovirus [sweepovirus] was identified for the first time in Uganda and the second time in Africa. The first full sequence of an African sweepovirus was obtained; it is a new virus and, like others, has a monopartite genome. Sweepoviruses have also been found in Tanzania and reconfirmed in Kenya. This and our work with sweepoviruses found in South and Central American countries have been brought together with other sequences in databases to show how sweepoviruses separate, with most East African samples falling within the 'Leaf curl' group. Most isolates, when co-infected with SPCSV, were not associated with more severe symptoms, the only viruses infecting sweetpotato not to be synergised. However, one isolate (collected in St Vincent Island) was synergised, inducing leaf curling symptoms in co-infected sweet potato and begging the question 'What difference enables this isolate alone to be synergised?'

All potyvirus isolates closely related to SPFMV were found to contain an extra overlapping gene in the P1 region of the potyviral polyprotein. The function of this gene is not yet known, but bio-informatic predictions suggest it may be an Ago-binding silencing suppressor.

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; amongst others, 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 controlling especially SPFMV

Subsequent to the project's submission but prior to the project's 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 gene silencing-based virus resistance and so synergising SPFMV. Development of SPVD following infection of RNase3-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. Recombinant RNase3 protein has been prepared using *Escherichia coli* for expression and affinity chromatography for purification but attempts to develop monoclonal antibodies failed.

Sectioning of SPFMV-infected plants and *in situ* hybridisation, immuno-localization and laser capture microdissection was used to confirm if meristem exclusion has a role in SPFMV resistance. SPFMV was 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. The breadth of RS-based resistance in sweetpotato is exemplified by its breakdown against diverse viruses during synergistic co-infection with SPCSV (see above), broken by its RNase3 gene and cooperating p22 gene. Therefore, the model plant *Arabidopsis thaliana* was transformed with the RNase3 gene, with an alanine mutant RNase3 gene that has lost its ability to cleave siRNA (a possible control for the RNase3 mutation) and with the p22 gene. Few transformants with p22 survived but several of those with RNase3 and its Ala mutant survived and expressed RNase3. SPFMV infected neither untransformed controls nor the transformants but *Tobaco mosaic virus* (TMV), *Turnip crinkle virus* (TCV) and *Cucumber mosaic virus* (CMV) all did. Both the RNase3 and its mutant Ala Arabadopsis were more susceptible to TMV and CMV than the parent untransformed line and it may be that

cleavage of siRNA by RNase3 is not key to its role in synergy. However, neither RNase3 nor mutant ala Arabidopsis showed increased symptoms so synergy is not strong. RNase3, its mutant Ala and the parent untransformed line appeared similarly susceptible to TCV.

The Ugandan strain of SPCSV which synergised SPFMV in sweetpotato only poorly had an RNase3 gene and a p22 gene that closely resembled ones reported in the virus gene base for strongly synergising isolates of SPCSV. However, qPCR revealed that there were apparently fewer copies of these genes in the poorly-synergising strain, along with all other genes in RNA1 so this entire genomic molecule may be suppressed.

3. Is resistance to SPCSV in sweetpotato an extreme of RS-based resistance or otherwise?

A mutant sweetpotato with an 'abnormal' array of siRNAs when infected by SPCSV was generated whilst attempting transformation with a hairpin' silencing construct targeting the 3'UTR of SPCSV in the Peruvian sweetpotato variety Huachano. Plants of this genotype artificially (graft) infected with SPCSV returned to healthy status and no SPCSV was detected when indexing these plants in *I. setosa*. In plants inoculated with SPCSV + SPFMV (which would normally result in SPVD), SPCSV was also eliminated and SPFMV titers returned to those occurring in a SPFMV-single infected plant.

4. Are sources of resistance effective in the field and their deployment?

DLP 3163, previously found to be resistant to SPCSV in Peru, was severely affected by SPVD when grown outside in the Ugandan Sweetpotato Program crossing blocks and laboratory tests confirmed its susceptibility to graft inoculation to Ugandan and Tanzanian SPCSV and therefore its unsuitability as parental material in East Africa.

It has been confirmed in glasshouse experiments in the UK using qPCR and graft-inoculation to *I. setosa*, and in a screenhouse in Uganda using ELISA that plants of some Ugandan varieties of sweetpotato, notably New Kawogo, revert to healthy following SPFMV infection. NASPOT 11 [Tomulabula], a variety bred from New Kawogo by participatory plant breeding, expressed this character very strongly, showing it is heritable. Few symptomless vines of New Kawogo and NASPOT 11 obtained from the field in Uganda were virus-infected, consistent with their reversion from infection. In time-course experiments, SPFMV titres in resistant varieties gradually declined to zero. As well as being consistent with RS-based resistance, it may provide a screening method for identifying resistant varieties.

NaCCRI developed supporting data for the release in Uganda of NASPOT 11, an SPVD resistant, high-yielding and otherwise superior sib of New Kawogo selected by farmer participatory selection in previous DFID-supported research. This is the first ever national release of a sweetpotato variety bred by PPB anywhere in the world. Farmers found that this variety satisfies a broad range of their requirements. A film was made of the process of selection: http://www.nri.org/work/tomulabula.html . NASPOT 11 has already been disseminated widely in Luwero district; farmers report improvements in their livelihoods, largely as a result of the creation of a new market for them - selling planting material to farmers and local NGOs.

Scientific and practical significance of the work carried out

The project has added considerably to the scientific knowledge of sweet potato viruses, especially in Africa. The cavemoviruses and the sweepoviruses had been particularly poorly characterized; this body of work now provides the bulk of current knowledge. African sweepoviruses seem to have been almost completely overlooked until now, yet even from current knowledge are clearly very common, probably throughout sub Saharan

Africa. According to work in the U.S.A., they are also cause >20% yield loss to individual plants and, economically, may even be the second most important virus affecting the African sweet potato crop. We have characterized one sweepovirus; there seem likely to be several others present.

The identification of a third southern African strain of SPCSV to complement the East and West African ones may complete the main evolutionary branches of this virus. The project has demonstrated that almost all viruses, including both RNA and DNA ones infecting sweet potato are synergised by wild type SPCSV, presumably through the suppression of plant-based RNA silencing by the viral RNase3 and p22 genes, and, since all the viruses are symptomless in the absence of SPCSV, confirms the efficacy of plant RNA silencing when it is not suppressed. The exception to being synergised is Sweet potato leaf curl Uganda virus and some other sweepoviruses – but interesting, at least one sweepovirus, from the Caribbean, is synergised. The isolate of the East African strain which does not synergise SPFMV in crops may present a considerable economic threat as well as being evolutionarily significant, as this represents a massive increase in its fitness, as signified by its dominance already in Busia District. It appeared to achieve this by wholesale suppression of RNA1, on which lie the RNase3 and p22 genes, an apparently unique mechanism. This is consistent with its reduced ability to suppress host plant RNA silence – to the detriment of SPFMV but apparently not to itself.

A sweetpotato clone with a unique pattern of siRNA was found which provided very high degree of resistance to wild type SPCSV.

In the absence of SPCSV, RNA silencing is so strong in certain Ugandan landraces that SPFMV is gradually eliminated from extending stems and these apparently provide virus-free cuttings. Plants with this attribute commonly have a low titre of SPFMV. This elimination has been followed by qPCR and by grafting to an indicator plant, *I. setosa*. Validation of PPB included release of a variety; as well as having many attributes obviously beneficial to farmers, it also has strong RNA silencing.

Principal conclusions and any opportunities arising from them.

1. We consider the main viruses affecting sweet potato crops in Africa have now been identified. Sweepoviruses still remain poorly described relative to their probable economic importance.

2. We recommend that a careful watch is kept over the situation in eastern Uganda where a SPCSV isolate occurs which, in the field, does not synergise SPFMV and does so only weakly in the laboratory. This attribute is expected to increase its fitness and hence its frequency.

3. Reversion from infection with SPFMV to healthy has been confirmed in African landraces. It is associated with a low virus titre when infected. African research institutes such as NaCCRI have the technical capacity to identify such genotypes; it should become part of the final screen before varietal release.

4. PPB has been validated for sweet potato. It selected for many beneficial farmer attributes but also selected for ability to revert – confirming that this is a very important character enabling survival of a variety under African conditions. We recommend that this breeding approach should be widely adopted.

5. A ‘new’ sweet potato clone highly resistant to SPCSV has been identified. It will be included in future breeding programmes.