DFID P*lant* S*ciences* R*esearch* P*rogramme*

FINAL TECHNICAL REPORT

R8031

GENETIC TRANSFORMATION OF RICE, POTATO AND COOKING BANANAS FOR NEMATODE RESISTANCE

Final Report DATE March 2006

TITLE OF PROJECT: - **Genetic transformation of rice, potato and cooking bananas for nematode resistance**

R NUMBER: R8031

PROGRAMME: Plant Sciences Research Programme

PROGRAMME MANAGER (INSTITUTION): University of Wales, CAZ, Bangor, and Wales.
SUB-CONTRACTOR (if relevant): The University of Leeds, Centre for Plant Sciences.

PROGRAMME PURPOSE: **The control of nematode pests in rice, potato and banana.**

EXECUTIVE SUMMARY

The need: Nematode damage to crops is estimated to be $125 billion (US) each year. We estimate this represents sufficient calories lost in Africa to meet the annual need of 50 million people. Nematode management is currently inadequate throughout the developing world and the problem intensifies as agriculture improves. Nematodes are small pests normally hidden from view in soil and they provide few symptoms to recognise the crop losses they cause. As a consequence growers are either unaware or underestimate the damage they impose. Few resource poor farmers receive extension advice. The least acceptable consequence of this is nematicide uptake. This is a trend when a problem is appreciated and the chemicals can be afforded in plantations or as agricultural development occurs. The risk these highly hazardous pesticides pose for both the environment and human health is evident from studies on banana plantations and field workers in Ecuador. Other approaches to control such as cultural rotation are often ineffective particularly because of wide host ranges or when poverty dictates a high dependence on certain, damaged subsistence crops. The low cost and environmentally safe approach to nematode control is natural resistance. Unfortunately appropriate resistance genes are usually not available in widely grown crops and there are few examples of successful breeding programmes for nematode control. There is little prospect of conventional resistance against a range of very different nematodes that damage crops such as banana, rice and potato.

The project: It developed global public goods that demonstrate and ability to control a wide range of nematode in three very different crops with dissimilar but damaging nematode problems. It was the main international project addressing this important food security issue. It drew on highly distinctive UK science skills. Its international importance was also centred in establishing that transgenic crop resistance can have a poverty focus and be both effective and fully biosafe. PSRP and DFID deserve credit for their foresight in supporting the development of the approach appropriately targeted at improving food security when other appropriate and effective solutions to the important problems of nematode control are unavailable. The outputs of this project have a general value for developing world agriculture and have are received considerable interest in many countries in Asia, Africa and S. America

The achievements: This project has demonstrated effective and a biosafe approach to nematode control using rice, potato and cooking banana as example crops. The approach relies on plant proteins (cystatins) that prevent digestion of dietary plant proteins by the feeding nematodes. Cystatins lack such effects on humans. They suppress the nematode's ability to grow, lay eggs and build to population levels that damage crops. Potato is readily transformed and been used as the crop of choice on which to develop the general value of the approach. It has established control against different nematodes. It showed that the safe protein could be restricted to a presence in roots or just where nematodes feed. We have shown that it also provides additive resistance achieving full control when combined with partial natural resistance. We have shown that a male sterile potato can be used to

evaluate efficacy in S. America so circumventing concerns about gene flow to wild relatives. Other cultivars were rendered male sterile by molecular means so that those cultivars favoured by subsistence growers could be deployed safely in the future. We showed that promoters that direct expression to just roots or where nematodes feed. This prevents more of these safe proteins occurring in the human diet than naturally present in common foods such as rice and maize seeds. Additional work funded by other donors (DEFRA) has demonstrated that the approach has no environmental impact on a wide range of non-target organisms. Work with rice has shown that nematodes that damage upland rice such as *Meloidogyne* spp (root know nematode) can be controlled and that clean gene technology enables the selectable antibiotic marker often associated with transgenic plants to be removed from the nematode resistant lines as a yet further contribution to biosafety. The project provided the first demonstration that East African Highland Banana can be transformed. Resistance to nematodes was demonstrated for this staple crop. There is high demand for this work and this part of the project continues as collaboration with Uganda funded by USAID.

Follow-up: DFID has developed a basis for controlling a wide range of nematode in many subsistence crops. Uptake is now required to ensure these global public goods of value reach the food insecure in West, East and Southern Africa, South Asia and the many other geographic regions with nematode problems in S. America and much of Asia. This is unlikely to be achieved without further support to the UK science base, as all the expertise required is not available in NARS or Advanced Research Institutes.

PRODUCTION SYSTEM: Rice, Banana and Potato

BENEFICIARIES: Tubers/ seeds/ plants provided royalty free for poor resource farmers in Uganda, India, Argentina, the transgenic plants/ seeds/ tubers could also be made available for other developing countries requesting the technology.

TARGET INSTITUTIONS: a) NARO, Kampala, Uganda; b) International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India but this was changed from India to China by DFID Central Research therefore and became c) Chinese Academy of Agricultural Sciences.

GEOGRAPHIC FOCUS: All eco-regions of Africa, Asia and South America

	Planned	Actual
START DATE:	March 2002	March 2002
FINISH DATE:	December 2005	March 2006
TOTAL COST:	£475,779	£473,030

1. Project Purpose:

Purpose: This project pursues a transgenic strategy for nematode resistance in three crops rice, potato and banana.
Organisation: It involved close collaboration between the Crop Genetics Department, The John Innes Centre (JIC, P. Vain and J. Snape) and The Plant Nematology lab. Centre for Plant Sciences, University of Leeds (CPS, Green and Atkinson). JIC was responsible for rice and banana transformation and development of clean gene technology for rice. All other aspects of the project were the responsibility of CPS. Co-ordination was achieved by informal e-mail exchange and by the meetings called at 6-monthly intervals by the PSRP manager (J. Witcombe).

Rice
This project developed nematode resistance in rice that addressed concerns associated with the release of transgenic plants in target countries. The aims of the project were to remove antibiotic resistance genes (or other marker genes) in rice plants (clean gene technology), to produce single copy transgenic plants with stable expression of transgenes (*Agrobacterium*-mediated transformation) that express anti-nematode proteins in roots only.

Banana
This project developed strategies to provide nematode resistance in East-African AAA cooking bananas. Emphasis was on control of *Radopholus similis*, the principal pest of this crop in most banana growing areas. Yield response studies of others suggest nematodes may reduce banana yields in Africa by 71 ± 16%. The project provided the first demonstration that transgenic East-African banana plants can be produced and losses prevented. This is now a subject of current effort by NARO (Uganda), CPS (UK) and KUL (Belgium) funded by the USAID ASBPII programme.
Potato
This project demonstrated that potato plant lines known to confer resistance against *Globodera* spp. (potato cyst-nematodes) are also effective against *Meloidogyne* spp. (root-knot nematodes). The aim was to demonstrate the general potential of the approach for nematode control. Mini-tuber based production was adopted. It underpins rapid transfer of lines to developing world partners. Male sterile potatoes were also developed for use to prevent gene flow where wild relatives of the plant occur (S & C. America). Male sterility was achieved by molecular means as contribution to prevent gene flow from a transgenic plants for cultivars favoured by subsistence growers. This is important where wild relatives of the potato occur (e.g. the Andes).

2. Outputs:

I: Rice: Main objectives

a. **Production of new additive construct for rice and banana for transformation at the JIC.**

Early in the project PsMTA-GUS-nos and ARSK-GUS-nos constructs were produced and transformed into rice by biolistic transformation. This was to allow assessment of the PsMTA and ARSK root preferential promoters (Lilley, Atkinson *et al.*, 2004, *Plant Biotechnology Journal,* 2, 3–12) in rice. Although these constructs were used in rice transformation, assessment of the transgenic lines at the JIC produced no lines worthy of closer study.
Two dual inhibitor constructs were made using a GO peptide linker from a galactose oxidase which is not cleaved in plants so ensuring a hybrid proteinase inhibitor is formed with two different activities (Urwin, Atkinson *et al*., 1988, *Planta*. 204, 472-479). In the first instance two new constructs were made control of the CaMV35S promoter and the ubiquitin (Ubi) constitutive promoters. The was to

achieve maximum expression and simplify detection at the western blot level while the potential of additive resistance was assessed. Root preferential promoter gives lower and more localised expression making detection of the inhibitor more difficult.

The two constructs differed in the cystatin they expressed. One carried the sequence of a maize cystatin and the second was a rice cystatin protein engineered to eliminate one amino acid (OcIΔD86). The maize cystatin was linked by GO to a squash aspartyl proteinase inhibitor normally expressed in its fruit (SQAPI). This construct is shown in Figure 1. The second construct was under control of CaMV35S and encoded for an OcIΔD86 and a serine proteinase inhibitor from cowpea seeds (CpTI). Both were constructed in the pGreen vector to allow clean gene technological rice plants to be produced (see later).

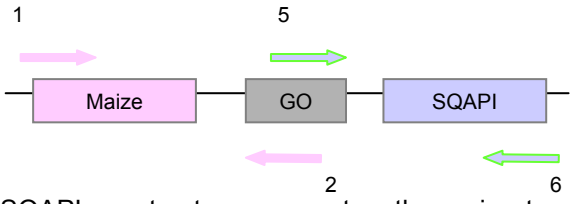


Figure 1: The Maize-GO-SQAPI construct was sown together using two sets of primers as seen above this then was ligated to the promoter and intron then cloned into the pGreen vector.

The construct was overexpressed in an *E.coli* expression vector pQE30 to assess whether or not both parts of the construct were working attached to the GO linker. Western analysis of the protein allowed detection of expression levels. The inhibitors were both expressed and Figure 2 provides an example for the maize cystatin-GO-SQAPI construct.

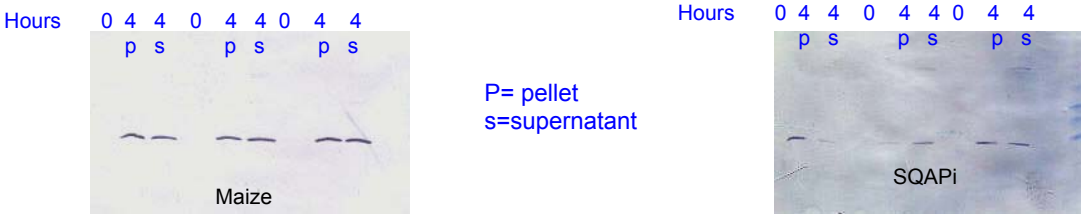


Figure 2: Western analysis of protein over expressed in *E. coli.* Expression is seen for both maize and SQAPI protein. This suggests that the GO linker is capable of delivering both proteins when expressed in the transformed plants.

b. **Production of rice plants containing defence genes against nematodes via high throughput*Agrobacterium* mediated transformation.**

The *Agrobacterium*-mediated transformation of *TUB:ubi: maize (native):nos* was carried out at the John Innes Centre by Dr Philippe Vain. The plants were then sent to Leeds University for molecular characterisation and bioassays against *Meloidogyne incognita*. The plants were analyzed by western blots. Their protein expression level was low but most plants were transgenic with expression confirmed at the RNA level. Southern blots and qPCR data established that one line studied (NN15) carried the preferred, single copy of the transgene while another line of interest carried two copies (NN31). The first two batches were bioassayed against *M. incognita* but the rice plants failed to flourish due to winter growth conditions prevailing when the containment trial was conducted. This resulting in a low multiplication of nematodes in control plants ensuing that the levels of resistance of transgenic lines could not be defined accurately. The trial was established again using PCR and RT-PCR to confirm they harboured and expressed the construct. The RT-PCR results established that the

majority of the plants were producing RNA for the transgene (Figure 3). DNA was not amplified from the RNA samples establishing the DNA contamination of samples had not occurred.
These plants were subjected to western analysis but very low levels of protein expression were detected. This is expected when using the tubulin promoter, as the level of expression is very low and localised within roots being particularly active in giant cells induced by *M. incognita.*

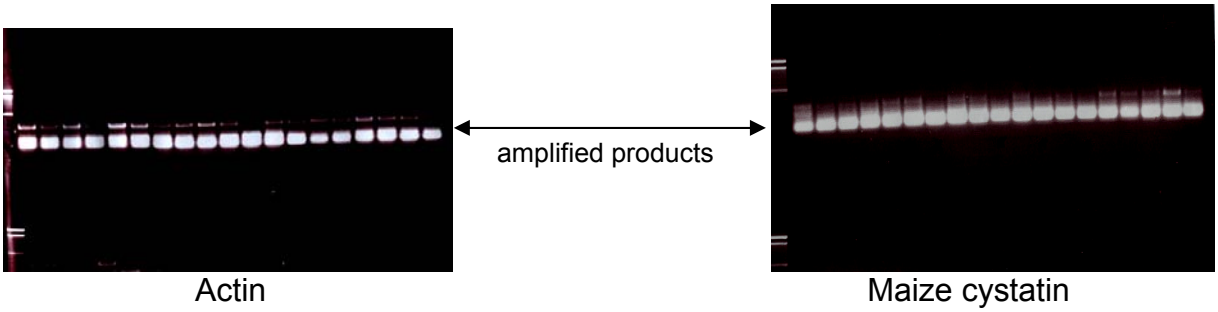


Figure 3. RT-PCR analysis of TUB-ubi-maize-nos of 19 transformed rice plants. Actin was amplified as a control to show equal RNA loading per lane. The transgenic plants are transcribing the maize cystatin sequence.

These plants were then taken onto bioassay against *M. incognita*. The plants on the third batch had very good light and temperature conditions that subsequently will allow good multiplication of the nematodes.

c. **High throughput testing of transgenic rice plants for nematode resistance**

Bioassays were carried out against *Meloidogyne incognita*. This is the major nematode pest of developing world agriculture (see earlier expert reports to DFID by others underpinning the establishment of this project). The results established few transgenic lines supported no egg production but the growing conditions did not support normal multiplication on the controls. Therefore a further bioassay was conducted with vigorous plants.

For the further trial, JIC provided T1 plants from 3 transformation events. The number of transgenes inserted at each transformation event is uncertain Therefore T1 seed was grown in CPS and selfed seed produced. The T2 should segregate and one progeny without the transgene would be expected in 4, 16, 64, 256 plants if there is 1, 2, 3 or 4, transgenes inserted at transformation. The frequency of PCR -ve plants suggests the three clones have 1, 2-3 and 3 genes respectively inserted at transformation. Quantitative PCR on the genomic DNA is being conducted at JIC on this T2 seed to determine copy number.

Different number of inserts provides different gene doses for the various heterozygote states and so a range of resistance levels may occur. The plants were examined statistically in a preliminary manner for the >100 PCR positive plants for one clone having 3 copies of the transgene. There is a highly significant negative skew even when smaller plants are excluded that may have been less successfully invaded by *M. incognita* (Figure 4). This normal curve analysis suggests there are more plants with no or few eggs than expected. This could be a refection of resistance from plants with high gene doses. This subset of plants suggest a very high level of resistance to *M. incognita* (i.e. >90% resistance).

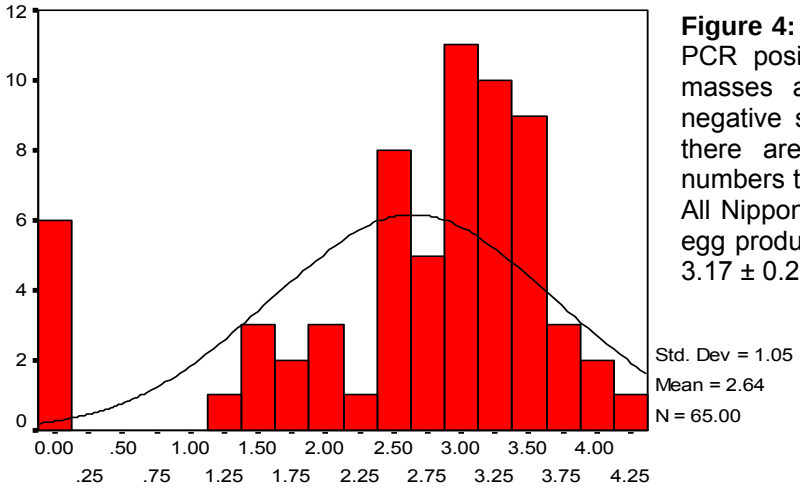


Figure 4: Histogram of $\log_{10}$ eggs/plant for all 65 PCR positive plants of one clone with foliage masses at harvest of >40g at harvest. The negative skew is highly significant (P<0.01, i.e. there are more plants with no or low egg numbers than expected for a normal distribution). All Nipponbare wildtype control plants supported egg production with a mean egg number of $\log_{10}$ 3.17 ± 0.23 eggs/plant; i.e. 1467 egg /plant).

The above analysis helped prioritise plants for more molecular characterisation. Plants were sub-categorised into those providing no, low or high *M. incognita* eggs at harvest. The plants were also subdivided into their foliage biomass groups at harvest and the smallest plants discounted from further analysis. The selection of plant sizes aims to exclude plants that seem to be resistant merely because they were too small at nematode challenge and so not attractive to the invading nematodes. The larger plants supporting production of no or few eggs/ plant are now being characterised to identify one or more resistant homozygote lines among them. Those homozygous lines showing resistance will be re-assayed to confirm that the main aim of this section of a high resistance levels in rice to *M. incognita* was achieved.

d. **Dissemination of nematode resistant rice plants.**

This was delayed by low expression levels in the first lines of interest limiting their utility as pioneer lines for field evaluation. JIC and CPS are seeking a funded collaboration to prepare and assist in a field trial of the lines of high value (Figure 4) once homozygote, resistant plants have been identified.

II: Banana: Main objectives:

a. Production of banana plants containing defence genes against nematodes

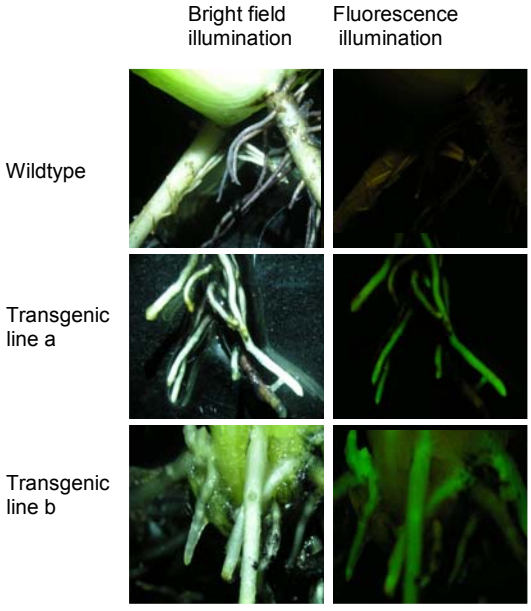


Figure 5: Expression of a fluorescent reporter gene encoding green fluorescent protein demonstrates that a protocol for transformation of Easter African Highland banana was achieved.

Transformation was achieved at the John Innes Centre by Dr Philippe Vain. The final report from JIC proves fuller details than this summary below. This work provided the first demonstration that East African Highland banana can be transformed. Initial transformation protocols were first established using green fluorescent protein (GFP) as reporter than could be easily visualised (Figure 5). The fluorescence of the plants was verified at both JIC and CPS.

Once banana transformation has been achieved with GFP, further lines were produced by biolistic transformation using Leeds constructs CaMV35S-Chicken egg white cystatin and CaMV35S-maize cystatin. Plants shown by PCR to carry the gene of interest were evaluated further for expression of mRNA (RT-PCR) and protein (western analysis; Figure 6). Transgenic lines that expressed the protein were challenged by *M. incognita* in containment at the Leeds University.

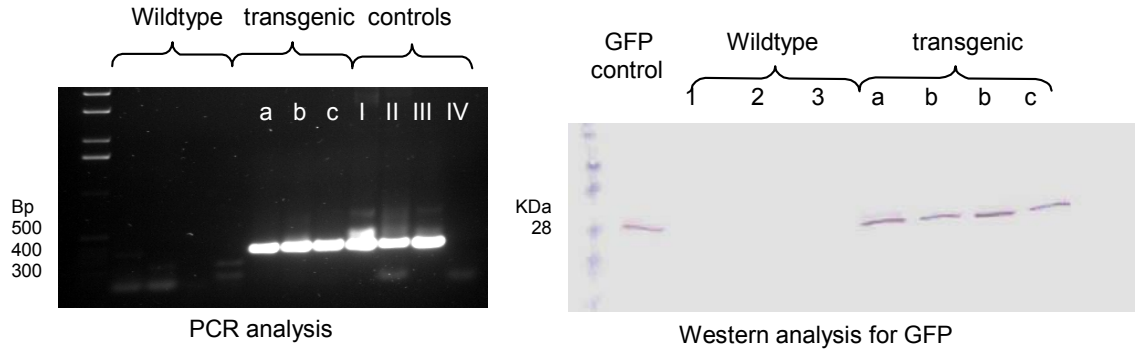


Figure 6. PCR detection of CEWc sequence in transformed banana lines (a-c) plus wildtype controls (1-4) and positive control lines from a potato transgenic line (I) and two *E. coli* clones (II, III) plus a negative control of water only (IV). The transgenic lines express GFP reporter protein (a-c) as does the GFP reporter banana (see Figure 4) but not wildtype plants. Western analysis involved 40µg total soluble protein per lane.

Testing of transgenic banana plants for nematode resistance in containment in the UK

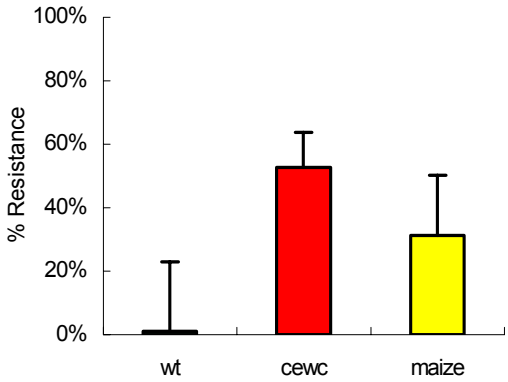


Figure 7: Partial resistance of two transgenic lines of East African Highland banana to *M. incognita* expressing either chicken egg white cystatin (CEWC) or maize cystatin in comparison to the wildtype (wt) of the same cultivar.

The two lines tested showed partial resistance to *M. incognita* (Figure 7). This was the first demonstration world-wide of a transgenic East African Highland banana. These banana lines expressed two different cystatins so establishing the approach. They results built on our first demonstration of any transgenic banana with a useful trait carried out in other work funded at CPS by Syngenta. The resistance to *M. incognita* in the two East African Highland banana cultivars was only moderate but the lines demonstrate that our goal is achievable.

b. Field-testing of transgenic banana plants for nematode resistance in Uganda.

The field trial testing on the Cavendish banana lines Ubi-OcIΔD86 and Tub-OcIΔD86 were not initiated, as Syngenta would not release these lines for research purposes although they were produced in collaboration between them and CPS. The lines made in the current programme have only a small amount of resistance to *M. incognita* (~50% in the best line). Lines have been multiplied at JIC and

are being evaluated for their resistance to *R. similis* in containment at CPS. If resistance over >50% is obtained they will be transferred to NARO in Uganda. This technology transfer is being funded at CPS by USAID. The lines will be used as prototypes and helped establish biosafe practises for transgenic field trials in Uganda authorised by their national biosafety committee.

Potato: main objectives

a. To assess impact of the transgenic potato lines against *Meloidogyne incognita*.

Due to space constraints, the containment trial with *M.incognita* was limited to two lines for each of the root preferential promoter constructs (see Lilley Atkinson et al 2004, *Plant Biotechnology Journal*, 2, 3–12 for details of these promoters). The lines chosen for *RPL16A* and *ARSK1* were those that had demonstrated the highest level of resistance against *Globodera* in a field trial. The containment trial against *M. incognita* was terminated 6 weeks after infection, to coincide with egg production, and at this stage no substantial tuber development had taken place. Multiplication of *M. incognita* on untransformed Desiree was > 100-fold (Pf/Pi = 117), which is consistent with the expected multiplication rate of this nematode over 6 weeks on potato. Resistance of the transgenic lines is expressed as a percentage reduction in the total number of viable eggs recovered from each transformed plant compared to the wild-type in Figure 7. All the transgenic lines supported significantly less eggs than the control ($P < 0.01$ or $P < 0.001$, see Figure 8). A transgenic line (T1) expressing the cystatin under control of the *TUB-1* promoter provided the highest level of resistance (67 ± 9%) compared with 59 ± 6% resistance for the previously characterized line using the CaMV35S promoter. This work demonstrates that potatoes showing resistance to *Globodera* spp are also resistant to *M. incognita*. Given that we have also established efficacy against Beet cyst nematode (*H. schachtii*), Reniform nematode (*Rotylenchus reniformis*) Burrowing nematode (*Radopholus similis*) it is evident that cystatin offers a defence effective against a wide range of nematodes. This is an important issue in the developing world given that nematodes are not visible to the naked eye and many resource poor farmers do not receive extension advice to identify the nematode pest. Growers only need to aware of that nematodes may be damaging their crops. It is now possible to control more than one nematode pest of the crop with crop resistance. These advantages are not shared by natural resistance approaches.

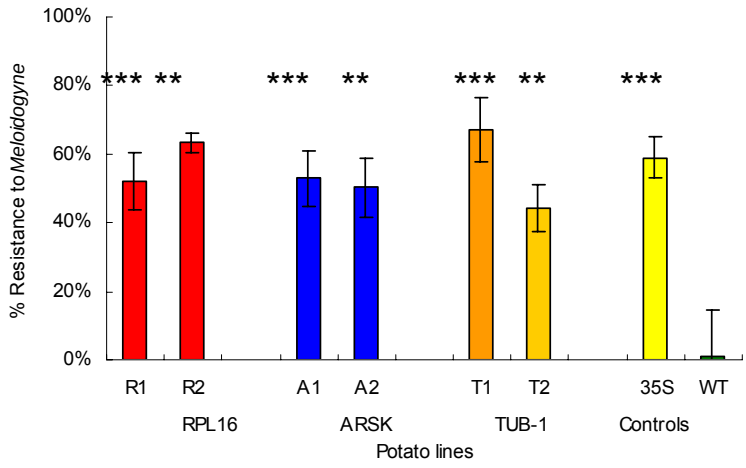


Figure 8: Transgenic resistance to *M. incognita* in containment of transgenic lines expressing the cystatin OclΔD86 from either a root preferential promoter or CaMV35S. Resistance of the transgenic lines is expressed as a percentage reduction in the total number of viable eggs recovered from each transformed plant compared to the wild-type. WT= wild-type untransformed cv. Desiree, 35S = a previously characterized line expressing OclΔD86 under control of the CaMV35S promoter to a level of 0.3% total soluble protein. $^{**}P < 0.01$, $^{***}P < 0.001$.

b. To produce biosafe potato lines using male sterile cultivars or by emasculation by molecular means

Use of a male sterile cultivar: Revolucion is the only potato cultivar of *S. tuberosum* andigenum cultivar series and/or hybrids between it and tuberosum cultivars that is male sterile. It therefore cannot spread transgenes via pollen to any cross-fertile wild relatives where they occur in C. and S. America (e.g. The Andes). We therefore transformed it to express the cystatin OcIΔD86. Transformation of andigenum series cultivars has only been reported on one previous occasion. Lines were generated that expressed the cystatin (Figure 9). Resistance to *Globodera pallida* is conferred on susceptible, male-sterile *Solanum tuberosum* cv Revolucion when it is transformed to express a cystatin. Values are means ± SEM and all are significantly different from the wildtype control at P<0.01 or P<0.001; Figure 10). Cluster analysis for phospholipids fatty acid analysis (37 compounds) and community level physiological profiles (31 substrates, BIOLOG Eco plates) establish that that expression of cystatins does not perturb soil microorganisms. This is contrast to measurable effects as consequences of a grower's free choice of crop or cultivar to grow (Celis, Green Atkinson *et al.*, 2004). This is the most recent of a series of publications in which we have established no unwanted environmental impact of the technology. It is safe for growers in the developing world to use. Our aim is that transgenic crops should have no greater environmental impact than the free choice that growers already make over which crop to grow.

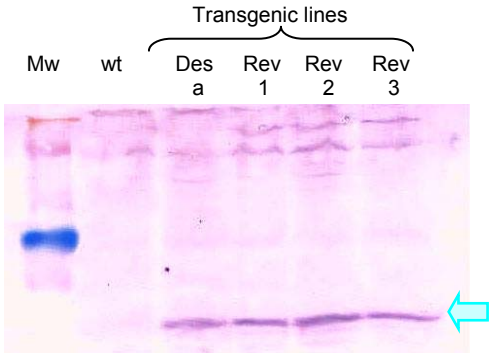
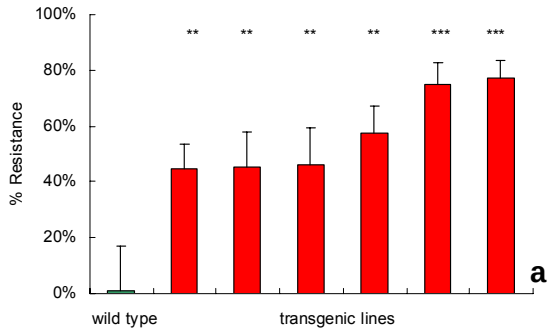


Figure 9. Three transgenic potato lines of cv Revolucion (Rev 1-3) were assessed by western analysis to determine expression of OcIΔD86 (arrowed) under control of the constitutive CaMV35S promoter. One transgenic line of cv Desiree (Des a) shown to provide >70% field resistance to *G. pallida* was used as a positive control



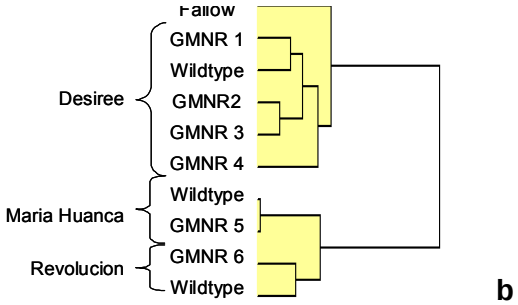


Figure 10 a) Demonstration of resistance to the important Andean nematode *Globodera pallida* achieved using a male sterile cultivar suitable for the Central Andean countries (**, P<0.001; **, P<0.01, published as Celis, Green, Atkinson *et al*., 2004). This helps prevent gene flow from the transgenic lines to the many wild relatives of potato in this geographical region; **b**) the GM nematode resistance (GMNR) we have developed has no effect on soil micro-organisms in contrast to the choice of potato cultivar grown in the soil. This conclusion is based on analysis of phospholipids fatty acids and sole carbon substrate utilization profiles (BIOLOG eco plates) to assess any change in microbial soil communities in which the plants were grown.

Emasculation by molecular means: dependence on the male sterile cultivar Revolucion does not meet the cultivar range required by the poor in Central Andes. We have explored the potential to

widen the range of cultivars in which the technology can be offered without risk of gene flow from pollen. We developed a male sterile Desiree (commonly grown in Bolivia) using a tapetal specific promoter and either a bacterial RNAse (barnase) used to generate sterility in plants before or a ribosomal inactivating protein (RIP) normally expressed in maize seeds (Figure 11). This is the first demonstration that a plant RIP commonly consumed in food has activity against plant cells. The approach has potential for biosafe GM vegetatively propagated crops in the developing world. This is appreciated by experts in the field, e.g. the manager of the USAID plant biotechnology programme (ABSPII).

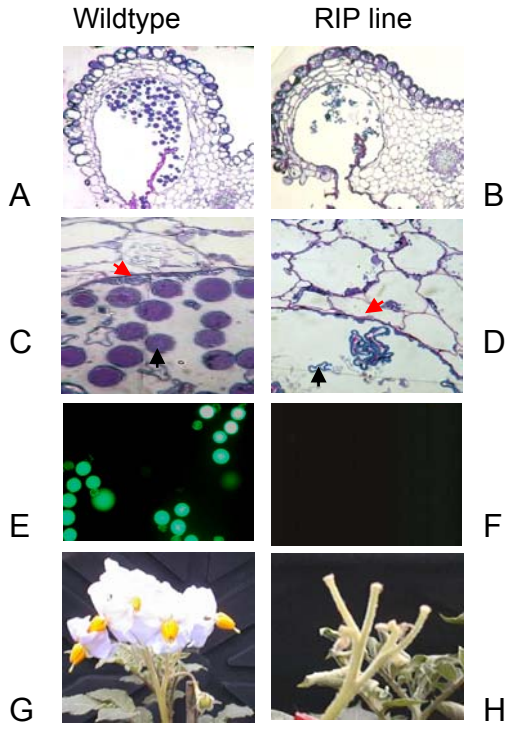


Figure 11: Male sterility of potato cv Desiree induced by expressing a RIP under control of a specific, tapetal specific promoter. Bright-field micrograph cross section through anther pollen sacs contrasting the tapetal cell layer (red arrow) and pollen grains (black arrow) of wildtype (**A, C**) with those in which male sterility has been induced (for a RIP expressing line) (**B, D**). **E**, viable pollen of a wild type plant taking up the vital fluorophore FDA; (**F**) non-viable pollen of the RIP line with one grain showing viability, (**G**) berry formation results for hand pollination using viable wild type pollen but (**H**) not that from the RIP line (published as Green, Atkinson *et al.*, 2005).

To assess additive resistance in potato.

This was achieved and published in Urwin, Green and Atkinson (2003). It shows that the addition of nematode resistance genes to R-genes found to be naturally resistant to certain nematodes give full and durable resistance (Figure 12). The approach has high potential for the many crops in which plant breeders have identified sources of resistance which do not completely suppress nematode multiplication and damage. It has potential for suppressing selection of virulent nematodes that overcome natural resistance. This is an established problem for both *Meloidogyne* and cyst nematodes.

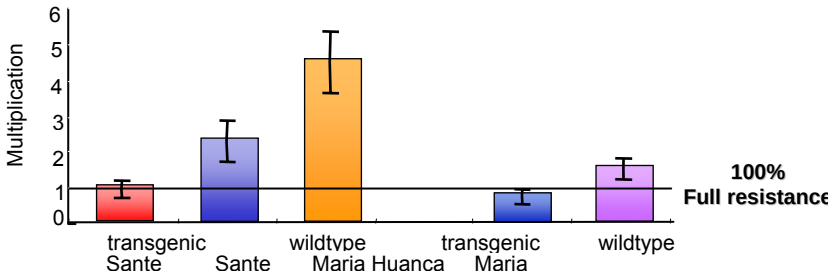


Figure 12. Mean resistance of transgenic potato lines in the field. Transgenic cvs. Desiree expressing either Oc-IΔD86 or sunflower cystatin under control of CaMV35S compared with values for the untransformed susceptible cv. Desiree and both transgenic lines of the partially resistant cv. Sante expressing CEWC in comparison to untransformed cv. Sante. The horizontal dotted line represents no multiplication on the crop. Multiplication on control Cv Desiree (fully susceptible was 20x).

Field-testing of transgenic potato lines for nematode resistance overseas.
Transgenic lines below were sent to the Institute of Vegetables and Flower, The Chinese Academy of Agricultural Sciences (CAAS), Haidian District, Beijing on the 8th May 2005. China was specified by DFID as the immediate partner for this work. This was another first for the PSRP research programme. We are told these potatoes are the first transgenic material to authorised by the Chinese Ministry of Agriculture for introduction to China (Figure 13) for evaluation there rather being produced with the country.

We have since held informal talks with Dr Rao of the Ministry of Biotechnology, India about their introduction to that country. Progress was also made in gaining permission for field trial in Argentina, which has all necessary biosafety regulations in place. However the project has ended before permission has been granted and we now lack funds for more trials. There is also interest from elsewhere in S. America and Africa to which we can no longer respond.


中华人民共和国
农业转基因生物材料入境审批书

Figure 13: Import licence of the Institute of Vegetables and Flower, The Chinese Academy of Agricultural Sciences, Beijing

In 2005, the tubers were transferred to CAAS, China and bulked there in preparation for containment and field trial in that country scheduled for 2006 (Table 1). We have provided resources and expertise to underpin that trial and visited China to discuss the trial and development of the collaboration in relation to nematode control in peri-urban agriculture.

	Cultivar		
Expression pattern	**Desiree (global)**	**Revolucion (S. America)**	**Maria Huanca (S. America)**
Constitutive expression	9/31, 35SCaMV-OclΔD86-nos	Rev2, 35SCaMV-OclΔD86-nos Rev10, 35SCaMV-OclΔD86-nos Rev16, 35SCaMV-OclΔD86-nos	MH10, 35S-CEWc-nos
Root specific expression	32/31, ARSK-OclΔD86-nos 31/35, RPL16-OclΔD86-nos 33/26, TUB OclΔD86-nos		
Untransformed controls	Wildtype	Wildtype	Wildtype

Table 1: Summary of transgenic and control lines provided as mini-tubers for evaluation in China

3. Contribution of Outputs to Project Goal.

Our goal was to modify crops of banana, rice and potato by molecular means to overcome the biotic constraints of nematodes. This was achieved in all three crops so establishing the potential of the approach for any transformable crop in the developing world with a nematode pest problem.

Potato provides the most advanced demonstration of the approach and its biosafety. The Institute of Vegetables and Flower, The Chinese Academy of Agricultural Sciences, Beijing is now evaluating the potato lines before wider deployment in that country. We would welcome the opportunity to transfer the lines to other countries able to evaluate them such as Argentina, India and Uganda. They have value in demonstrating resistance of the potato crop. Scientists in both India and Uganda has appreciated their more general significance for showing that *Meloidogyne* spp can be controlled on a wide range of crops by this approach. Control of this nematode was set as a target by reports commissioned by DFID before the RNRRS programme was initiated.

The banana component of the project has also achieved success. It has delivered the first transgenic bananas with nematode resistance in East African cooking bananas. Although resistance in these lines is lower than we obtained before with a Cavendish variety, they demonstrate that the transformation was achieved. The production of more lines with resistance will be achieved particularly if further support is secured. Aspects of the project have been taken up by USAID ABSPII. This funding NARO Uganda, KUL Belgium and CPS, Leeds to develop transgenic nematode and fungal resistant bananas for Uganda. CPS is carrying out capacity building with Uganda through the current grant and with additional funds from USAID and Rothamsted International/Gatsby Africa Fellowship scheme. The latter paid for 6 months training of Dr Josephine Namaganda on biosafety in relation to transgenic banana. A second Ugandan may spend 4 month in CPS from Makerere University Uganda receiving training in food safety. USAID is establishing a confined field trial site and helping NARO establish a transformation facility and a capacity to test Ugandan-produced transgenic bananas. The DFID project has laid the foundation on which that progress will be made. We will provide Uganda with all useful banana lines generated in this project.

The rice-based work has achieved success with well over 80% resistance to *Meloidogyne incognita* using a maize cystatin under the control of a root-preferential promoter. This work is of importance in demonstrating that *M. incognita* can be controlled. This is the major nematode pest of a wide range of crops in all eco-regions of Africa and much of South Asia. Progress was delayed by biolistic transformation, which has until recently been essential for some crops including rice. This led to the unforeseen problem of silencing within rice. This is not an issue using *Agrobacterium*-mediated transformation and so the work has transferred to that approach now it has been established for rice. This approach linked to clean gene transformation system was established for rice. This system has been shown achieve resistant plants without a selectable marker. Plants generated by this approach are been trialled successfully against root-knot nematodes and homozygous lines are being identified prior to dissemination to NARS or Advanced Research institutes.

4. Publications:

Green J., Fearnehough M.T. and Atkinson H.J. (2005) Development of biosafe, genetically modified *Solanum tuberosum* (potato) cultivars with an emphasis on issues concerning it centre of origin. *Molecular Breeding* 16: 285-293.

Atkinson H.J., Green J., Vain P., Pinto Y., Koyama M. and Snape J.W (2005) Genetically modified crops can contribute to pathways out of poverty. *Aspects of Applied Biology* 75: 109-114.

Atkinson, H.J., Grimwood, S.J., Johnston K.A., Green, J. (2004) Prototype demonstration of transgenic resistance to the nematode *Radopholus similis* conferred on banana by a cystatin. *Transgenic Research*, **13**, 135-142.

Celis, C., Scurrah, M., Cowgill S.E., Chumbiauca, S., Green, J., Franco, J., Main, G., Kiezebrink, D.T., Visser, R.G.F., Atkinson, H.J. (2004) Environmental biosafety and transgenic potato in a centre of diversity for this crop. *Nature*, **432**, 222-225.

Amoussou P-L., Ashurst J., Bridge J., Green J., Jones M., Koyama., Snape J.W. and Atkinson H.J. (2004) Broadly based resistance to nematodes in the rice and potato crops of substance farmers. Plant Sciences Research Programme Highlights and Impact. Crop Transformation 9-13.

Urwin PE, Green J. and Atkinson H.J. (2003) Expression of a plant cystatin confers partial resistance to *Globodera*, full resistance is achieved by pyramiding a cystatin with natural resistance. *Molecular Breeding* 12:263-269.

Green J, Vain P, Fearnehough M.T, Worland B, Snape J.W. and Atkinson H.J. (2002) Analysis of the expression patterns of the *Arabidopsis thaliana* tubulin-1 and *Zea mays* ubiquitin-1 promoters in rice plants in association with nematode infection. *Physiological and Molecular Plant Pathology,* 60: 197-205.

5. Internal Reports:

Annual end of year reports 2002, 2003 and 2004

6. Other Dissemination of Results:

6a) Research Presentations and posters

Atkinson, H.J.; Cowgill, S.E., Green, J., and Kiezebrink, D.T. (2004) *Progress and challenges in developing transgenic nematode resistant crops for subsistence agriculture*. Invited presentation at XXVII ESN International Symposium Rome June 2004.

Green J., Fearnehough M.T. and Atkinson H.J. (2004) Globodera *spp. Resistant, male sterile potato plants for biosafe uptake in the crops centre of diversity.* Poster presented at XXVII ESN International Symposium Rome June 2004.

Atkinson HJ, Green J, Vain P, Pinto Y, Koyama M and Snape JW (2005) *Genetically modified crops can contribute to pathways out of poverty*. AAB Pathways out of Poverty Conference, Cambridge, September.

Atkinson HJ (2006) *Recent Progress on Molecular Approaches to Novel Crop Resistance against Nematodes,* Brazilian National Nematology Conference, February 2006 (Invited Speaker).

Atkinson HJ (2006) *Recent Progress on Molecular Approaches to Novel Crop Resistance against Nematodes.* Invited international speaker to the S. Korean Government Conference on Applied Genomic (Seoul, June 2006).

Atkinson HJ (2006) *Prospects for transgenic control of nematodes on banana.* Invited International Speaker to the annual conference of the Organisation of Tropical America Nematologists, (Costa Rica, June 2006).

6b) Research Seminars to Institutes on biosafe, nematode resistant crops for the developing world

These have been presented widely. Examples include: **Africa**, Uganda (NARO, 2004 and 2005), **Asia**, Chinese Academy of Sciences (3 institutes, 2003 and 2005), Chinese Academy of Agricultural Sciences (2 institutes, 2005), Zheijang University (2003); **South America**, Peruvian Ministry of Agriculture, Lima, Peru (2005); **UK**, Dept Plant Sciences U. Oxford (2005), SCRI (April 2005) and Rothamsted (2003).

6c) Examples of capacity building activities

- A two week residential course was developed and organised for African Scientists on biosafety issues surrounding transgenic crops (2004; funded by DFD Environment Dept).
- Atkinson HJ was chair of an international, external review commissioned by The International Potato Centre (a CGIAR institute) on strategies for development and deployment of genetically engineered potatoes and sweetpotatoes (Lima, June 2005)
- Host of a Rothamsted International Africa Fellow (Dr Josephine Namaganda, NSRO) to develop skills relating to biosafe uptake of GM banana (July-December 2005).
- Host for an Indian Government Fellowship to develop transgenic approaches for nematode control on Indian cereals (Dr Uma Rao, Indian National Agricultural Research Institute, January-July 2006).
- PhD training award (NERC Dorothy Hodgkin to Mr Dong Wang (a Chinese national on reducing the environmental risks and hazards of crop production by biosafe use of transgenic crops, using China as a case study (2005-2008).

7. Follow-up indicated / planned:

If strategic research, can / will the results be followed through into the adaptive phase?

This is being achieved in an incremental way with support from USAID and by developing relationship with NARO (Uganda), The Indian National Institute of Agricultural Research and The Chinese Institute of Agricultural Research. There is a need for further funding to assure rapid and certain progress and we hope for involvement in the new DFID research programmes including the eco-regional programmes (e.g. East Africa and S. Asia) and post-RNRRS programmes. We have many potential collaborators and USAID would be interested in discussing cost sharing for such work with DFID. We are concerned about the lack of intentional donors for research to benefit the poor of C. Andes.

8. Name and signature of author of this report.

Prof. Howard John Atkinson