Biotechnology helps engineer resistance to rice nematodes

RIU

Validated RNRRS Output.

An effective and biosafe method of controlling nematode worms in rice is now ready for trials in rice-growing countries. Nematodes are a serious pest in a wide range of crops and the technique is generating a lot of interest, particularly in South Asia, Africa and South America. Tested on a wide range of crops in the USA, China and Uganda, the technology is already being used in both developed and developing-world countries. It has great potential for agriculture in India and China where governments strongly support biotechnology as a way to rapidly raise food production.

Project Ref: **PSP19:**
Topic: **1. Improving Farmers Livelihoods: Better Crops, Systems & Pest Management**
Lead Organisation: **University of Leeds, UK**
Source: **Plant Sciences Programme**

Research into Use
NR International
Park House
Bradbourne Lane
Aylesford
Kent
ME20 6SN
UK

Geographical regions included:

Africa, Asia, South America,

Target Audiences for this content:

Crop farmers,

Document Contents:

Description

PSP19

A. Description of the research output(s)

1. Working title of output or cluster of outputs.
In addition, you are free to suggest a shorter more imaginative working title/acronym of 20 words or less.

Genetically engineered resistance to rice nematodes

2. Name of relevant RNRRS Programme(s) commissioning supporting research and also indicate other funding sources, if applicable.

Plant Science Research Programme (ID code PSP0032).

3. **Provide relevant R numbers**
(and/or programme development/dissemination reference numbers covering supporting research) along with the institutional partners (with individual contact persons (if appropriate)) involved in the project activities. As with the question above, this is primarily to allow for the legacy of the RNRRS to be acknowledged during the RiUP activities.

PSRP grants R6453 R6948 R7294 R8031

Institutional partners on DFID PSRP grants
R8031

Prof. HJ Atkinson, Centre for Plant Sciences, University of Leeds. LEEDS LS2 9JT, tel 0113 3343 2900 e-mail h.j.atkinson@leeds.ac.uk

Dr Philippe Vain and **Prof. John Snape**, John Innes Centre, Colney Lane, Norwich. NR4 7UH, UK. Tel: +44 (0) 1603 450000 , Fax: +44 (0) 1603 450023, E-mail: philippe.vain@bbsrc.ac.uk & john.snape@bbsrc.ac.uk

Dr. Tushemereirwe, Kawanda Research Institute (KARI), NBRP/NARO, P.O. Box 7065, Kampala, Uganda

Current funding of the biotechnology but not its application to developing world includes BBSRC Agrifood committee and its Crop Science Initiative plus NERC Dorothy Hodgkin Studentship for crop environmental biosafety (Chinese national).

4. Describe the RNRRS output or cluster of outputs being proposed and when was it produced? ***(max. 400 words)****.*
This requires a clear and concise description of the output(s) and the problem the output(s) aimed to address. Please incorporate and highlight (in bold) key words that would/could be used to select your output when held in a database.

This project has demonstrated effective and a biosafe approach to nematode control of rice pests. 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. Specific promoters have been developed that ensure the cystatin is expressed where nematodes feed in roots and not throughout the whole plant. 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.
The plant biotechnology strategy adopted involved *Agrobacterium*-mediated transformation and high-throughput molecular screening of clean gene progenies. This was carried out at the JIC by Dr P. Vain. The plants were then sent to Leeds University for molecular characterisation and bioassays against *Meloidogyne incognita* (the man

target species according to expert reports for DFID completed by others that underpinned establishment of this project). Lines were confirmed by Southern blot and qPCR analysis. Single copy transgenic lines plus some containing two or three copies of the cystatin gene were identified. All of these lines were generated using the clean gene technology developed at JIC (see RIU dossier 18 on "clean gene technology"). This approach segregates the selectable marker used in selecting lines of interest after the first generation of transformed rice. The aim is to develop homozygous lines expressing the nematode resistance but not the selectable marker. This is often resistance an antibiotic so its elimination aids biosafety in terms of both food and environmental safety. Lines contained different numbers of inserts provide different gene doses and therefore a range of resistance levels. Many plants had a very high level of resistance to *M. incognita* i.e. >90% resistance obtained and require only final selection of homozygous seeds before donation within the RiUP.

In other aspects of the work, root preferential promoters have been developed for rice to limit activity to where nematode parasites of roots occur. In other work, (BBSRC funded) additive resistance has been developed and is available for RiUP.

The work provides a key example internationally of how transgenic technology can be adapted to meet the needs of the poor. The outputs of this project have a general value for developing world agriculture and have are received considerable interest in many countries in S. Asia, Africa and S. America.

5. What is the type of output(s) being described here?
Please tick one or more of the following options.

Product	***Technology***	***Service***	***Process or Methodology***	***Policy***	***Other Please specify***
x	*x*				

6. What is the main commodity (ies) upon which the output(s) focussed? Could this output be applied to other commodities, if so, please comment

The main commodity in which the approach is relevant for this proforma is rice. There has been accompanying work on both potato (RIU dossier 21) and banana (RIU dossier 20) within the RNRRS grants developing this output. The technology is applicable to all crops for which transformation has been achieved. The benefits extend indirectly to other crops when they are grown in close rotation with rice. Nematodes such as *Meloidogyne* damage a wide range of crops and multiplication on rice can re-dispose follow on crops to damage. This also applies to control of *Pratylenchus* spp grown rice particularity when other cereals are the follow-on crop.

7. What production system(s) does/could the output(s) focus upon?
Please tick one or more of the following options. Leave blank if not applicable

Semi-Arid	***High potential***	***Hillsides***	***Forest-Agriculture***	***Peri-urban***	***Land water***	***Tropical moist forest***	***Cross-cutting***
	x	*x*					

8. What farming system(s) does the output(s) focus upon?
Please tick one or more of the following options (see Annex B for definitions).
Leave blank if not applicable

Smallholder rainfed humid	Irrigated	Wetland rice based	Smallholder rainfed highland	Smallholder rainfed dry/cold	Dualistic	Coastal artisanal fishing
	x	x	x			

*9. How could value be added to the output or additional constraints faced by poor people addressed by clustering this output with research outputs from other sources (RNRRS and non RNRRS)? (**max. 300 words**). Please specify what other outputs your output(s) could be clustered. At this point you should make reference to the circulated list of RNRRS outputs for which proformas are currently being prepared.*

These outputs could be clustered with others addressing the safe use of biotechnology for nematode control in potato and banana (R6380, R6743, R7548 and R8031). These project share a similar approach and hence some resources. This biotechnology approach could also be stacked with that for RYMV resistance (R7415, R7548). There is also potential to combine with other plant biotechnology approaches such as marker assisted breeding (R6673, R7080, R7434, R7435, R8200 and R8089).

The approach could also be clustered with other approaches such as participatory breeding for rice improvement (R8221, R8099, R6826, R7657, R7434, and R8099).

Nematode resistance could be provided in context of other approaches to enhance rice productivity. Priming of upland rice (R7438) is of interest as nematodes damage young plants. There has been some preliminary work between Leeds and Dr D. Harris (Bangor) on the interactions between rice priming and nematodes.

Validation

B. Validation of the research output(s)

***10. How** were the output(s) validated and **who** validated them?*
*Please provide brief description of method(s) used and consider application, replication, adaptation and/or adoption in the context of any partner organisation and user groups involved. In addressing the "who" component detail which group(s) did the validation e.g. end users, intermediary organisation, government department, aid organisation, private company etc... This section should also be used to detail, if applicable, to which social group, gender, income category the validation was applied and any increases in productivity observed during validation **(max. 500 words)**.*

Methods of Evaluation
The efficacy of the approach were examined thoroughly in containment trials and some of the work for RNRRS has been published in peer review journals and detailed in reports to DFID (see below)

Afolabi SA, Worland B, Snape JW and Vain P (2004) A large-scale study of rice plants transformed with different T-DNAs provides new insights into locus composition and T-DNA linkage configurations. *Theoretical and Applied Genetics* 109: 815-826
Amoussou P-L., Ashurst J., Bridge J., Green J., Jones M., Koyama., Snape J.W. and Atkinson H.J. (2004).

Plant Sciences Research Programme Highlights and Impact. Crop Transformation 9-13.
Atkinson H.J., Green J., Vain P., Pinto Y., Koyama M. and Snape J.W (2005) *Aspects of Applied Biology* 75: 109-114.
Green J, Vain P, Fearnehough M.T, Worland B, Snape J.W. and Atkinson H.J. (2002) *Physiological and Molecular Plant Pathology,* 60: 197-205
James V, Worland B, Snape JW and Vain P (2003) The effects of matrix attachment regions (MARs) on transgene expression levels and stability in rice. In *Advances in Rice Genetics* G. S. Khush, D. S. Brar, B. Hardy eds. pp538-541.
James VA, Worland B, Snape JW, Vain P (2004) Development of a standard operating procedure (SOP) for the precise quantification of transgene expression levels in rice. *Physiologia Plantarum* 120: 650-656.
James VA, Worland B, Snape JW, Vain P (2004) Strategies for precise quantification of transgene expression levels over several generations in rice. *Journal of Experimental Botany* 55: 1307-1313
James VA, Avart C, Worland B, Snape JW and Vain P (2002) The relationship between homozygous and hemizygous transgene expression levels over generations in populations of transgenic rice plants *Theoretical and Applied Genetics* 104: 553-561.
Vain P, Afolabi AS, Worland B and Snape JW (2003) Transgene behaviour in populations of rice plants transformed using a new dual binary vector system : pGreen / pSoup. *Theoretical and Applied Genetics* 107: 210-217.
Vain P, Harvey A, Worland B, Ross S, Snape JW & Lonsdale D (2004) The effect of additional virulence genes on transformation efficiency, transgene integration and expression in rice plants using the pGreen/pSoup dual binary vector system. *Transgenic Research* 13: 593-603
Vain P, V James, Worland B and Snape JW (2003) Transgene structure and expression in a large population of rice plants and their progeny. In *Advances in Rice Genetics* G. S. Khush, D. S. Brar, B. Hardy eds. pp550-551.
Vain, P. Worland, B. Clarke, M.C., Richard,G. Beavis, M. Liu, H. Kohli, A., Leech, M., Snape, J., Christou, P. & Atkinson, H.J. (1998), *Theoretical and Applied Genetics* 96, 266-271.
Vain P, Worland B, Clarke MC, Beavis M, Kohli A, Leech M, Snape J, Christou P and Atkinson H (1997) Expression of an engineered cysteine proteinase inhibitor (oryzacystatin-IΔ86) in transgenic rice plants. *International Rice Research Newsletters* 22: 9-10.

Who was involved?

The University of Leeds designed constructs and conducted all glasshouse trials. The Cereal Transformation group of JIC was responsible for development of transformation, and clean gene technology plus production and selection of lines of interest for detailed study.

The outputs from the programme were then validated by peer review before publication (see references cited above). The technology for control of nematodes was also validated by demonstrating its efficacy in other crops such as potato (see dossier 21) and bananas (see dossier 18). The technology is being evaluated in a wide range of crops by USDA, Chinese Academy of Agricultural Sciences, and USAID. There are a range of others interested in developing further this evaluation process.

Evidence of demand

Our studies for other crops suggest growers welcome the new power of biotechnology and would like to judge the benefits. This pro-science and practical approach is likely to prevail among many developing world growers.

We have not studied this issue for rice as it have been thoroughly covered by many others included the DFID chief scientist (G. Conway) and Michael Lipton possibly the UK's most eminent social scientist in this field and a member of the Nuffield, Bioethics commission. The key points are that transgenic crops can raise yield potential and field yields on those difficult low progress areas least touched by the Green Revolution (Conway 1989; *The Doubly Green Revolution : food for all in the 21st century.* London. Penguin). Upland rice would fall very firmly in that category. Lipton (2001 *Journal of International Development,* 13, 823-846) is of the view that rice is one crop which can be improved to cheapen or improve stable crops eaten by the poor. The need is traits that have poverty focus and resistance to pests are given as example traits of value. The outstanding issue is the availability of such traits as public goods. More recently it has been reported that poorer nations are attempting to develop public research in this area (Cohen, 2005, *Nature Biotechnology*, 23, 27-33). Rice is the most frequently transformed crop in this context.

A number of developing world research institutes has shown interest in the nematode resistance developed with in the RNRRS/PSRP. They include: WARDA , the Chinese Academy of Agricultural research (Prof Qu) and IRRI In India, recent interaction has been with the Indian National Agricultural Research Institute in Delhi. This fits well with there funding to Leeds from FCO/Indian government for collaboration between Leeds and IARI. Discussions with nematologists at IARI, confirmed severe nematode problems in much of the northern India's rice/wheat cropping system. We are exploring the development of a relationship between the IARI and DFID's India desk.

DFID/PSRP is the only international programme that has developed nematode resistance. It represents a distinct UK contribution to global public goods available royalty-free to the Research into Use Programme. Clearly all the factors are in place that to bring nematode resistance successfully into use.

11. ***Where and when*** *have the output(s) been validated? Please indicate the places(s) and country(ies), any particular social group targeted and also indicate in which production system and farming system, using the options provided in questions 7 and 8 respectively, above* ***(max 300 words)****.*

The outputs have not yet been evaluated in the developing world as RNRRS came to an end coincidently with plants generating seeds for such work. IARI are keen start the process of research into use.

Current Situation

C. Current situation

12. ***How and by whom*** *are the outputs currently being used? Please give a brief description* ***(max. 250 words****).*

The seeds from the RNRRS programme are safely stored at JIC in a designated facility until they and Leeds University can gain further funds to deliver these important outputs.

13. ***Where*** *are the outputs currently being used? As with Question 11 please indicate place(s) and countries where the outputs are being used* ***(max. 250 words).***

Products are not currently being used in rice because of the end of RNRRS funding. The technology has been widely taken up (see earlier) in both developed and developing world countries. Subsequent development of UK agriculture is continuing with strong BBSRC support from both Agrifood committee and The Crop Science Initiative. These outputs could readily flow into the RiUP.

14. What is the scale of current use? Indicating how quickly use was established and whether usage is still spreading ***(max 250 words).***

Output products are not currently being used in rice. The technology is being evaluated in wide range of crops by USDA (USA), CAAS (China), NARO (Uganda). A visit in Later October 2006 is to develop a basis for transfer of rice and potato to India for evaluation and use their. The technology has been field trialled in the UK but uptake has been delayed by attitudes to such plants with Europe.

15. In your experience what programmes, platforms, policy, institutional structures exist that have assisted with the promotion and/or adoption of the output(s) proposed here and in terms of capacity strengthening what do you see as the key facts of success? ***(max 350 words).***

The biotechnology to be deployed should be shown to be fit for purpose and fully biosafe when expressed in rice. This requires experiment under the field conditions of different rice production systems in the targeted country. This is necessary to establish the benefits and future potential the technology to various stakeholders before uptake is progressed. A careful and thorough analysis of the benefits as well as the apprehensions surrounding deployment of biotechnology is requited to ensure they can benefit the poor.
A government that has a pro-biotechnology attitude is essential. The governments of both India and China are committed to facilitating substantial rather than incremental approaches to enhancing food production. We will focus on India. It also has a progressive national biosafety committee/system. It has a number of NARS with a good science capacity and hence the ability to adopt the global public goods and reduce them to use on rice in India. It also has an extension service network able to explain the potential of biotechnology and be involved in its dissemination. It has good contacts with growers willing to support scientific improvements whose successes favour diffusion to further growers. The input of social scientist is important to support the process, assess uptake and determine safe dispersal to the informal seed system so extending to the poorer sections of the grower community.

Current Promotion

D. Current promotion/uptake pathways

*16****. Where*** *is promotion currently taking place? Please indicate for each country specified detail what promotion is taking place, by whom and indicate the scale of current promotion* ***(max 200 words).***

Output products are not currently being used in rice. The technology is in pre-commercial development in USDA labs in Hawaii, it is being trialled at Institute of Vegetables and Flowers (CAAS), Beijing. Agreements are in place to extend this to Soybean in China (CAS, Beijing) and Coffee in Brasil (University of Campos dos Goytacazes, Brasil). Other technology being developed with BBSRC funding is being developed commercially by Monsanto and could be available for use in rice by DFID on a royalty-free basis.

17. What are the current barriers preventing or slowing the adoption of the output(s)? Cover here institutional issues, those relating to policy, marketing, infrastructure, social exclusion etc. ***(max 200 words)****.*

17.1. The PSRP maintained a balanced programme of crop improvement and planned translation research phase for the rice plants that was curtailed by the end of the RNRRS programme.

17.2. Funding to enable uptake of the lines of interest by a developing world institute able to carry out field trials in a country with necessary biosafety regulations for their uptake. IARI in India offers that opportunity and an enthusiasm to be involved. There needs to be short phase to confirm the efficacy of nematode resistance in seed derived from plants tested before to confirm stability of expression before adoption by resource poor farmers is considered.

17.3. Biosafety issues particularly related to concerns of outcrossing of the crops to other rice crops or wild relatives need to be addressed where there is a risk.

17.4. There is a lack of appreciation of the importance of nematodes. Most international research institutes lack activity in this area results in the opportunity not being prioritised relative to fields of work for which more expert knowledge is available. IARI is one NARS that is an exaction to this generalisation. This of course adds to the argument that DFID should commit to completely the processor making these important global goods available to the poor.

18. What changes are needed to remove/reduce these barriers to adoption? This section could be used to identify perceived capacity related issues ***(max 200 words).***

A key need for development of plant biotechnology is to appreciate that large gains can be achieved if continuity of effort is maintained to realise them. This type of work does not lend itself to a short-term approach and the ending of the RNRRS programme ended continuity on nematode resistant rice. The translational research period requires similar consistency of effort. The issue is that many options offered to DFID lack the power to make substantial improvement to food security. Although they are relatively inexpensive, they will have little positive impact on world food security. In contrast, plant biotechnology has high research power and can deliver a wide range of benefits to very many people through the simple act of seed distribution. The impact and need for plant biotechnology is evident from the investment that China is making using its strong science base. India would benefit from support from the UK science base. The distinct DFID funded nematode resistance rice is clearly an opportunity make substantial improvement to rice production in that county and elsewhere is S. Asia later.

19. What lessons have you learnt about the best ways to get the outputs used by the largest number of poor people? ***(max 300 words).***

19.1. Demonstration of efficacy of the trait under the farming conditions of the poor
19.2. Scientists in a NARS committed to the opportunity and well resourced to complete the translational research and so develop “home grown” technology
19.3. Effective farmers’ schools or other approaches to engage with growers on the benefits and to allay apprehensions etc.

19.4. Local ownership of the biotechnology so the "home grown" approach can be developed
19.5. A positive environment for biotechnology from a range of stakeholders including politicians and media.
19.6. A national government with commitment to plant biotechnology and with all necessary legislation in place and active. India meets these criteria

---

Impacts On Poverty

E. Impacts on poverty to date

20. Where have impact studies on poverty in relation to this output or cluster of outputs taken place? This should include any formal poverty impact studies (and it is appreciated that these will not be commonplace) and any less formal studies including any poverty mapping-type or monitoring work which allow for some analysis on impact on poverty to be made. Details of any cost-benefit analyses may also be detailed at this point. Please list studies here.

There have been a number of studies by eminent scientists and social scientists on the general issue of plant biotechnology and its benefits for the poor. Examples are listed below. The specific issue of the benefits from nematode resistance have been considered (Atkinson H.J. *et al.*, 2005, *Aspects of Applied Biology* 75: 109-114 and Atkinson H.J. *et al.* ,2001, *Trends in Biotechnology* 19, 91-96). The extent of need in terms on nematode loses to rice is defined in answer to question 22.

There have been both general considerations. Examples include:
Cohen JI (2005) Poorer nations turn to publicly developed GM crops, *Nature Biotechnology*, 23, 27-33.
De Groote H., Mugi, S., Bergvinson, D and Odhiambo, B (2004) Debunking the myths of GM crops for Africa: The case of Bt maize in Kenya. http://www.biw.kuleuven.be/aee/clo/euwab_files/degroote2004.pdf
Thirtle, C, Beyers, L., Ismael, Y Piesse, J (2003) Can GM-Technologies Help the Poor? The Impact of Bt Cotton in Makhathini Flats, KwaZulu-Natal *World Development*, 31, 717–732
Lipton M. (2001), Reviving Global poverty reduction, what role for genetically modified plants, *Journal of International Development,* 13, 823-846.

21. Based on the evidence in the studies listed above, for each country detail how the poor have benefited from the application and/or adoption of the output(s) ***(max. 500 words):***

- *What positive impacts on livelihoods have been recorded and over what time period have these impacts been observed? These impacts should be recorded against the capital assets (human, social, natural, physical and, financial) of the livelihoods framework;*
- *For whom i.e. which type of person (gender, poverty group (see glossary for definitions) has there been a positive impact;*
- *Indicate the number of people who have realised a positive impact on their livelihood;*
- *Using whatever appropriate indicator was used detail what was the average percentage increase recorded*

The principal uptake of plant biotechnology to-date in India is for Lepidopteran resistant cotton using *cry* genes (Bt). Extensive work in than country identifies how losses from insects are such that the value of the approach in cost saving to USA growers does not provide a paradigm for India. Here the levels of damage are more severe and there are less control options (Qaim & Zilberman, 2003, *Science* 299, 900-902). This analysis can be extended to nematode control. In this case, there are no options for the grower apart from reducing the intensity of cropping or rotating away from their rice crops to less favoured alternative. In addition, most growers are unaware of the damage caused and do not even attempt to take rectifying action. Therefore, the *a priori* case is that nematode resistance is exactly the sort of trait that will benefit resource poor farmers if made available to them without adding costs. It has the potential to control nematode without change to favoured cropping practises.

To date, no GM rice has been released / commercialized worldwide. The impact of GM rice on poverty is therefore difficult to assess. However, insect resistant B.t. rice is at a pre-commercialisation stage in both Iran and China (C. James, 2005, Global status of commercialized biotech/GM crop, ISAAA brief 34). The golden rice (http://www.goldenrice.org/) fortified with pro-vitamin A is also in the pipeline for release in Asia. Second generation golden rice plants containing c30µg provitaminA per gram of endosperm protein (i.e. 60g of golden rice now meets daily need) are currently grown at IARI (India).

Environmental Impact

H. Environmental impact

24. What are the direct and indirect environmental benefits related to the output(s) and their outcome(s)? ***(max 300 words)***

More agrochemicals are used on rice than on all other crops combined in the major rice-producing countries of Asia. Many of the compounds are outmoded chemicals banded from others part of the world. Nematicides are not commonly applied to rice although this use could grow as development occurs. The real issue is that alternatives to chemical control are needed that do not reduce the intensity of rice production or impose unwanted change to agricultural practices. Biotechnology has the potential to deliver alternative approaches to chemicals that growers can depend upon. There are therefore important what in shifting the mind set of many growers from an inclination to be pesticide dependent when resources allow to an equally effective but environmentally benign basis for crop protection and improvement.

This could include direct benefits from the application of the technology or policy action with local governments or multinational agencies to create environmentally sound policies or programmes. Any supporting and appropriate evidence can be provided in the form of an annex.

25. Are there any adverse environmental impacts related to the output(s) and their outcome(s)? ***(max 100 words)***

The approach will have no adverse environmental impact. All plants to be released will be biosafety and the removal of the selectable maker via clean-gene technology (see RIU dossier 18) will meet apprehension of some in relation to use of antibiotic or herbicide resistance as selectable markers.

26. Do the outputs increase the capacity of poor people to cope with the effects of climate change, reduce the risks of natural disasters and increase their resilience? ***(max 200 words)***

The outputs will enhance the ability of poor people to cope with droughts associated with climate change. The principal, direct consequence of nematode damage is to stunt root systems. This ensures plants are less able to obtain water and nutrients from soil. Common symptoms of attack are wilting and mineral deficiencies. This issue is very apparent for rainfed, upland rice. The effect is evident for other crops to which the plant technology could be applied later once biosafety is assured.
