Clean-gene technology has promise for safe genetically-modified crops

RIU

Validated RNRRS Output.

An easy and efficient way to develop genetically-modified crops that are 'biosafe' is now available. People worldwide are reluctant to accept genetically-modified foods. They are afraid that they might contain genes, such as those resistant to antibiotics or herbicides, which could be harmful. The clean-gene technology has great potential for Asian and African research programmes that aim to improve rice by genetic methods. It can also be readily used to improve crops grown by poor farmers in China, India and South Africa. Not only important staples, such as maize and wheat, but also orphan crops, such as millet, cowpea, sorghum and many fruits, nuts and vegetables could be improved by this technology. And laboratories in Asia, Africa, the USA and the UK are already using this process.

Project Ref: **PSP18:**
Topic: **1. Improving Farmers Livelihoods: Better Crops, Systems & Pest Management**
Lead Organisation: **John Innes Centre, UK**
Source: **Plant Sciences Programme**

Document Contents:

Description

Research into Use
NR International
Park House
Bradbourne Lane
Aylesford
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UK

Geographical regions included:

China, India, UK, USA,

Target Audiences for this content:

Crop farmers,

PSP18

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 rice free of selectable marker gene.

Working title/acronym: clean-gene technology

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

Plant Science Research Programme (ID code PSP0031).

The Rockefeller Foundation (New York, USA) provided additional matching funds to R7548 in order to support the grant and the University fees of the two PhD students involved in this programme. Dr. Abolade Afolabi (from Nigeria) successfully defended his thesis on the development of clean-gene technology in rice at the University of East Anglia (UK) in 2003.

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.

Plant Science Research Programme grants R7548 and R8031.

Institution directly involved in the project activities:
- John Innes Centre

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***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.*

The RNSS output is a **technology** called "**clean-gene**" that allows the production of **genetically modified** (GM) plants free of selectable marker gene (such as antibiotic or herbicide resistance genes) therefore improving significantly their **biosafety**. The clean-gene technology was developed over the 2000-2003 period using rice as a model species with high relevance to the developing world. However, it is a **generic approach** which can be applied to all sexually propagated plant species when undertaking genetic modification. Its principle is to transform plants using two separate vectors, one carrying the transgene of interest (desired additional trait) and the other carrying the selectable marker gene (useful to select the transgenic plants during the transformation process but unwanted afterwards). When the two vectors are delivered using a bacterium called *Agrobacterium tumefaciens* they integrate, in roughly half of the cases, at different locations in the plant genomes and therefore can be segregated from each other at the next generation. Around two percents of the rice progeny plants produced by clean-gene technology are free of selectable marker gene at the first generation providing an easy and efficient way to produce GM crop plants with improved biosafety.

In the past, the presence of selectable marker or reporter genes in transgenic plants represented an important constrain to the dissemination and acceptance of GM crops in the developing and in the developed world. By removing this constrain the output of this programme greatly facilitates the use of transgenic approaches to meet the needs of the poor.

***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				

***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 to this proforma is rice, however, clean-gene technology is a generic process that can be used during the genetic modification of all sexually propagated plant species. This includes important crops for subsistence farmers such as maize and wheat but also orphan crops such as millet,

cowpea, Sorghum as well as sexually propagated tree crops and vegetables.

***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*	*x*	*(x)*			*(x)*	

x : clean-gene technology use in rice
(x) : clean-gene technology use in other sexually propagated *plant species*

***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*	*x*	*(x)*		

x : rice farming systems
(x) : other systems where GM crops/trees could be used

***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.*

The output being a technology and not a product, it should be clustered with other outputs from transgenic research programmes aiming at crop improvement in the developing world. This includes outputs from RNRRS sources described in proforma dossiers No19 (“Genetically engineered resistance to rice nematodes”) and No21 (“Genetically engineered resistance to potato nematodes”) as well as R7415 project (“Transgenic resistance to Rice Yellow Mottle Virus in rice”). This also includes outputs from non-RNRRS sources focusing on GM crop research in National Research Programmes or CGIAR centres such as IRRI, CIMMYT etc…

The clean-gene technology can be used directly by many Asian and African research programmes aiming at improving rice through genetic transformation. Great added value could be obtained by using a clean-gene approach to transform locally adapted Asian and African rice cultivars as well as other crops species important for poor and subsistence farmers. The clean-gene technology can be used readily to improve the type of GM crops (such as insect resistant *B.t.* cotton and *B.t.* maize) currently grown by poor farmers in China, India and South

Africa and therefore can make a direct contribution across poverty groupings.

There is also a potential to combine the clean-gene technology outputs with other plant biotechnology and classical breeding approaches such as (*i*) marker assisted breeding described in proforma dossier No22 (focusing on rice) and (ii) participatory breeding described in proforma dossiers No1, 2, 6, 10 and 12 (focusing on rice).

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)**.*

The output provides biosafe technology to the international scientific community interested in using transgenic approaches for crop improvement. Scientists are the primary users and beneficiaries from the clean-gene technology. GM crops with poverty focus traits and available as public goods could have a strong impact on poverty across social groups, gender and income categories. The evidence for demand and the scope of beneficiaries from GM crops can be cross-referenced in proforma dossiers No19 to 21 using transgenic approaches to improve nematode resistance in rice, banana and potato.

How were the outputs validated?
The efficacy of the clean-gene technology has been validated through peer-reviewed publications in international journals:

Afolabi SA, Worland B, Snape JW and Vain P (2005) Novel pGreen/pSoup dual-binary vector system in multiple T-DNA co-cultivation as a method of producing marker-free (clean gene) transgenic rice (*Oryza sativa L.*) plant. African Journal of Biotechnology 4:531-540.
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.

In addition clean-gene binary vectors for plant genetic transformation have been distributed free of charge through material transfer agreements (MTAs) to scientists in developing countries (see paragraph below for details).

Who validated them?
The John Innes Centre was responsible for the development and initial assessment of the clean-gene technology. The University of Leeds quantified the resistance to nematodes of GM rice plants produced using clean-gene technology (proforma dossier No19).

The referees from international journals validated the scientific data relative to the clean-gene technology. In addition, the 2004 article of Dr. Abolade Afolabi (Nigerian PhD. Student funded by R7548) in Theoretical and Applied Genetics has already been cited six times during the first 10 months of 2006 indicating impact in the scientific community.

The scientist that requested the clean-gene vectors for use in their own research programme further validated the technology. This includes scientists from developing countries (Dr. Changyin Wu, China – Dr. Bharat Chattoo, India) but also scientists from developed countries working on research programmes focusing on the developing world (Dr. Amitabh Mohanty, USA). The clean-gene concept can also be used with other binary vector systems and many articles using modified versions of the clean-gene principle have been published.

Additional validation
The clean-gene technology would not have been developed without the initial work on transformation technology development (PSRP R6121 and R6148) and the ongoing efforts on understanding transgene integration and behaviour in rice (PSRP R8031). This research has been extensively validated through peer-reviewed publications in international journals:

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.
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, 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.
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, 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, James VA, Worland B and Snape JW (2002) Transgene behaviour across two generations in a large random population of transgenic rice plants produced by particle bombardment. Theoretical and Applied Genetics 105: 878-889.
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, Worland B, Kohli A, Snape JW, Christou C, Allen GC and Thompson WF (1999) Matrix attachment regions increase transgene expression levels and stability in transgenic rice plants and their progeny. The Plant Journal 18: 233-242.

Kohli A, Griffiths S, Palacios N, Twyman R, Vain P, Laurie D and Christou P (1999) Molecular characterization of transforming plasmid rearrangements in transgenic rice reveals a recombination hot-spot in the CaMV35S promoter and confirms the predominance of microhomology dependent re-combination. The Plant Journal 17: 591-602.
Kohli A, Gahakwa D, Vain P, Laurie P and Christou P (1999) Transgene expression in rice engineered through particle bombardment: molecular factors controlling stable expression and transgene silencing. Planta 208: 88-97.
Vain P, Worland B, Kohli A, Snape J and Christou P (1998) The green fluorescent protein as a vital screenable marker in rice transformation. Theoretical and Applied Genetics 96: 164-169.
Arencibia A, Gentinetta E, Cuzzoni E, Castiglione S, Kohli A, Vain P, Leech M, Christou P and Sala F (1998) Molecular analysis of the genome of transgenic rice (Oryza sativa L.) plants produced via particle bombarbment or by intact cell electroporation. Molecular Breeding 4: 99-109.
Kohli A, Leech M, Vain P, Laurie D and Christou P (1998) Transgene organization in rice engineered through direct DNA transfer supports a two-phase integration mechanism mediated by the establishment of integration hot-spots. Proc. Natl. Acad. Sci. USA. 95: 7203-7208.

These 12 articles have been **cited 341 times** in the international literature up to and including October 2006 (ISI web of science), attesting to their high impact.

***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)**.*

Where have the outputs been validated?
Clean-gene technology has been validated either through literature citation or request for binary vectors using Material Transfer Agreements (MTAs).
Literature citations: scientists from Belgium, USA, Australia, Denmark & England.
Request of clean-gene vectors via MTAs: scientists from India, China & USA.

When have the outputs been validated?
Clean-gene technology base been validated through scientific publications, citations and MTA requests over the 2004-2006 period.

Current Situation

C. Current situation

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

How are the outputs currently being used?

Clean-gene technology is being used to produce GM plants free of selectable marker genes following the methodology described in scientific publications (Afolabi *et al.* 2004, 2005) and using the freely available clean-gene binary vectors for plant transformation that we have developed. The clean-gene technology is being integrated into molecular breeding programmes in order to improve GM crop biosafety. This could rapidly benefit the type of GM crops (such as insect resistant *B.t.* cotton and *B.t.* maize) currently grown by poor farmers in China, India and South Africa.

The proforma dossier No19 ("Genetically engineered resistance to rice nematodes") provides an example of how clean-gene technology was used to produce GM rice plant resistant to nematodes and free of selectable marker genes.

By whom are the outputs are currently being used?

The international scientific community is currently using the outputs. Laboratories in Asia and Africa have been using the clean-gene technology as demonstrated directly by binary vector requests and indirectly by literature citations. For example, Dr. Changyin Wu (China), Dr. Bharat Chattoo (India) and Dr. Amitabh Mohanty (USA) are using our clean-gene binary vectors for crop transformation. Within the DFID PSRP programmes, Prof. Howard Atkinson (UK) has used a clean-gene approach to develop biosafe GM rice with improved resistance to nematodes for the benefit of developing countries (see proforma dossier No19).

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).

The principle of the clean-gene approach is being used internationally by the scientific community to develop plant molecular breeding programmes with improved biosafety. The clean-gene vectors are being used the following laboratories:

- Microbiology and Biotechnology Centre (MS University, Baroda, India)
- National Key Laboratory of crop improvement (Huazhong Agricultural University, China)
- Spring Harbor Laboratory (Cold Spring Harbor, USA)
- The Centre for Plant Sciences (University of Leeds, UK) has used clean-gene technology to produce GM rice plants resistant to nematodes (proforma dossier No19).

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

The first article on clean-gene technology was published in 2004. The adoption of the technology has taken around one year as demonstrated by the growing number of citations in 2006 and the continuing requests for clean-gene binary vectors for plant transformation since 2005. This dynamic is particularly good for this area of science as it generally takes years to implement changes in crop transformation programmes. Producing GM plants free of selectable marker is likely to become the international standard for both industry and academia and the contribution of the clean-gene technology in this area is likely to greatly increase. PSP0032 research programme has already implemented this strategy to produce clean-gene GM rice plants resistant to nematodes (dossier No19).

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 following factors have contributed to the promotion and adoption of the clean-gene technology:

- Statutory biosafety regulations, national and institutional policies on the release of GM crops have promoted the development of clean-gene approaches.

The publication of the clean-gene technology in peer-refereed international journals has strongly contributed to the scale and speed of dissemination.

Articles in web-based biotechnology journals aiming at developing countries such as the new agriculturalist online http://www.new-agri.co.uk/05-2/focuson/focuson7.html have also promoted adoption.

Additional web-based information provided by the institutions (John Innes Centre, DFID PSRP etc..) involved in this work:

http://www.research4development.info/caseStudies.asp?ArticleID=176
http://www.dfid-psp.org/Research/CropTrans4.html
http://www.jic.ac.uk/staff/philippe-vain/clean-gene.htm

Availability of clean-gene binary vectors for plant transformation.

Presentation of the clean-gene technology at International meetings, especially those in Africa and Asia:

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 conference, September 22nd-23rd , Cambridge, UK.

Vain P, Green J, Worland B, Derevier A, Snape JW and H. Atkinson (2005) Clean gene technology to produce marker-free rice plants containing cystein proteinase inhibitor genes against nematodes. EWAC Meeting. June 27th – July 1st. Prague, Czech Republic. S3-7.

Afolabi AS, Worland B, Snape JW and Vain P (2003) Development and understanding of new clean gene technology in rice. 7th International Congress of Plant Molecular Biology. June 23-28th. Barcelona, Spain. S10-95.

Afolabi AS, Snape JW and Vain P (2002) Development and understanding of new clean gene technology in rice. John Innes Centre Annual Science Meeting 2002. October 31st- November 1st. Norwich, UK.

Afolabi AS, Snape JW and Vain P (2002) Development of Agrobacterium-mediated clean gene (marker free) technology for rice transformation using a novel dual binary plasmid pGreen/pSoup. RF-NARO Conference on Biotechnology, Breeding and Seed Systems: research output that reaches farmers. Entebbe, November 4-7th. Uganda. p20.

Afolabi AS, Worland B, Snape JW and Vain P (2002) pGreen/pSoup dual binary vector in Agrobacterium-mediated co-transformation show good promise for generating marker free transgenic rice. 2nd Biennial Regional Rice Research Review (4Rs 2002) Meeting. April 9-12th. WARDA Headquaters, M'be/Bouake, Ivory Coast.

Afolabi AS, Snape JW and Vain P (2001) Development of Agrobacterium-mediated clean gene technology for rice transformation. John Innes Centre Annual Science Meeting 2001. November 1-2nd. Norwich, UK.

Vain P, James V, Worland B and Snape JW (2000) Transgene structure and expression in a large population of rice plants and their progeny. 4th International Rice Genetics Symposium, October 22-27th. IRRI, Philippines, p363.

The biotechnological solutions should be answering local needs and have strong biosafety records. This requires an assessment under field conditions in different locations, using different production systems and over several years by National Programmes. Further diffusion to growers via participatory breeding schemes can further contribute to the evaluation and ensure that transgenic plants are fit for purpose. The attitude of local governments towards biotechnologies is also a key factor for success.

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).

The clean-gene technology is promoted worldwide through peer-reviewed scientific publications and web-based information (see sections B and C of this dossier). The proforma dossier No19 (“Genetically engineered resistance to rice nematodes”) provides examples of promotion and uptake pathways for GM crops with improved biosafety produced via clean-gene technology.

***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)**.*

There are no barriers to the adoption of the clean-gene technology as the know-how and transformation vectors are freely available. The clean-gene approach does not bring additional intellectual property right constrains to existing transgenic strategies for crop improvement.

However, there are numerous factors that currently prevent the adoption of GM crops into agriculture, particularly regulatory and societal concerns about biosafety. Clean-gene technology aims to improve this situation by better biosefaty. The dissemination and commercialisation on GM crops, food, feed and products in Europe and Africa has been significantly slower than in America and Asia. In many African countries the absence of biosafety regulation and of means to implement them has further prevented the release of GM crops. This also has prevented adequate monitoring of GM plants potentially originating from food aid. Clean-gene technology could help in improving public and regulatory body acceptance by significantly improving the biosafety of GM crops.

The end of the DFID PSRP programme in 2006 has stopped the planned testing of nematode resistant GM rice plants produced by clean-gene technology (proforma dossier No19 “Genetically engineered resistance to rice nematode”) in developing countries.

***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).***

No changes are required for the adoption of the clean-gene technology by the international scientific community. However, many changes are needed to remove or reduce the barriers to the adoption of GM crops in Africa and to a lesser extend in Asia. Helping African countries to develop and implement biosafety regulations is a pre-requisite. Developing transgenic strategies with a strong poverty-focus (*i.e.* with relevant traits in relevant species) and a public-good approach are also key. Evaluation of the GM crops by participatory breeding after initial testing by National Programmes could help to demonstrate the benefits to poor or subsistence farmers. Maybe more consideration should also be given to the voice of developing world farmers who would like to explore for themselves the potential benefits of biotechnologies to improve their livelihood. The clean-gene technology could promote acceptance of GM crops through the improvement of their biosafety.

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).***

The primary users and beneficiaries of the clean-gene technology are the international scientific community concerned with GM crop improvement. The best ways to optimise use has been through quality and reproducibility of the technology. The proforma dossier No19 (“Genetically engineered resistance to rice nematodes”) provides examples of the lessons learnt in the most efficient ways to get GM rice produced via clean-gene technology used by the largest number of poor people.

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.*

The output provides enabling technology to the scientific community using genetic modification for crop improvement. Clean-gene transgenic rice plants have been trailed in containment for many years (see proforma dossier No19 “Genetically engineered resistance to rice nematodes”) but dissemination to developing countries has been stopped at the end of the DFID PSRP programmes in 2006.

To date, no GM rice has been released / commercialized worldwide. The impact of GM rice (clean-gene or not) on poverty is therefore difficult to assess post-release. However, insect resistant GM rice is at a pre-commercialisation stage in both Iran (500-1000 farmers) 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.

Many studies have also analysed in more general terms issues of plant biotechnology and its benefits to the poor:
- Cohen JI (2005) Poorer nations turn to publicly developed GM crops, Nature Biotechnology, 23, 27-33.
- 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.
- Toenniessen GH, O'Toole JC and DeVries J (2003) Advances in plant biotechnology and its adoption in developing countries. Current opinion in biotechnology 6:191-198.

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):***

To date, no GM rice has been released / commercialized worldwide, therefore only pre-commercialisation data are available for rice. In China, large scale pre-production trials of GM hybrid rice, staring in 2001, showed yield increases of approximately 4 to 8%, plus a saving of 17 kg per hectare in pesticides along with a labour saving of 8 days per hectare resulting in an overall increase in net income per hectare of $80 to $100 (C. James 2005).

The socio-economical and environmental impact of other GM crops - such as insect resistant *B.t.* cotton and *B.t.* maize - currently commercialised in developing countries - such as China, India or South Africa - has been the subject of many studies (Carl E. Pray et al. 2002 The Plant Journal 31:423-430 ; http://www.isaaa.org/). It is estimated that China has enhanced its farm income from biotech cotton by $4.2 billion in the 1997-2004 period. In 2005, 6.4 million small farmers were growing *B.t.* cotton in China. However benefits of a different type of *B.t.* cotton in India have been less clear, highlighting that GM approaches should be tailored to local environments (such as high pest and pathogen pressures), agronomic practices and needs.

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)***
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.

Clean-gene technology significantly improves the biosafety of GM crops by avoiding the presence of selectable marker genes – such as antibiotic or herbicide resistant genes – in genetically engineered plants. The absence of

antibiotic resistance genes (coding for kanamycin or hygromycin resistance) eliminates the risk of horizontal gene transfer to bacteria and therefore the risk of building up resistance to antibiotics (with therapeutic value) in micro-organisms. The absence of herbicide resistant genes (when used as a selectable marker gene) eliminates the risk of gene transfer to wild plant relatives and therefore prevents the development of "super-weeds".

Producing GM plants free of selectable markers is likely to become the international standard for both industry and academia and the contribution of the clean-gene technology in this area is important. PSP0032 research programme has already implemented this strategy to produce GM rice plants resistant to nematodes and free of antibiotic or herbicide resistant gene (proforma dossier No19).

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

The clean-gene technology has no adverse environmental impact. By avoiding the presence of selectable marker genes in GM crops the clean-gene technology considerably reduces the potential impact of GM crops on the environment and limits the risk assessment to the gene of interest present in the plant. An example of environmental impact of GM rice plants resistant to nematodes and produced by clean-gene technology can be found in proforma dossier No19.

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 output is an enabling technology to produce GM crops with improved biosafety and therefore, it does not have a direct effect on issues related to climate change. However, GM crops produced by clean-gene technology can make a significant contribution to increase the adaptability of plants to their environment, including climate change. Resistance to biotic (pests and pathogens) and abiotic (environmental) stresses are important avenues for biotechnology research. Insect resistance (using the *cry* genes from *B.t.*) is an example of GM trait deployed in Asia and Africa that provides lasting and significant help to cope with pest pressure. Dossier No19 details how nematode resistant GM rice produced by clean-gene technology can contribute to the capacity of poor people to cope with the effects of climate changes. The ever increasing reliance on plants for food, feed, fibre and fuel in the developing world makes a compelling case to use all the available approaches – conventional or biotechnological – to improve sustainable crop production.