BRIEFING PAPER: THE UCT/PANNAR GENETICALLY ENGINEERED MAIZE RESISTANT TO MAIZE STREAK VIRUS

19 SEPTEMBER 2007

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INTRODUCTION

There have been several reports in the media recently about the development of the first genetically engineered crop developed and tested solely by Africans.[1,2] Scientists at the University of Cape Town and colleagues at the South African seed company, PANNAR Pty Ltd have reported on the development of a transgenic maize variety resistant to maize streak virus. This is touted as a major advance for "African agricultural biotechnology that should contribute to a substantial improvement in African maize yields".[8]

In southern Africa, maize is the dominant staple food[4] and per capita consumption exceeds 100kg.[3] Maize consumption in Zambia, Zimbabwe and South Africa is highly influenced by government policy, which prioritises maize above all other crops, resulting in very few commercial substitutes', maize being the staple of low income groups, the bulk of the urban populations, in South Africa in particular.[4] The media reports on the UCT/Pannar project stresses that part of the objective is to provide seed that will be sold at a minimal profit to subsistence farmers, thus removing the objection that GE technology is principally profit-driven.[2] This paper outlines our understanding of the UCT/Pannar project and progress thus far and raises questions and concerns about the future of the maize streak resistant maize.

BACKGROUND TO THE PDR MSV MAIZE

Maize streak virus (MSV) causes a disease of maize that produces yellow lines or streaks on the plant that reduces the plant's ability to grow and to fill cobs.[5] MSV, a geminivirus spread by the leafhopper vector, wreaks havoc on infected maize crops. Only growing leaves can be infected and the younger the plant at infection the greater the impact of the virus on the plant as a whole. The extent of MSV pathogenicity is easily measured because MSV populates mesophyll cells within precisely defined chlorotic lesions of infected maize leaves and the extent of the chlorotic surface area of infected leaves is positively correlated with the total quantity of viral DNA within the leaf.[6]

Geminiviruses have circular single stranded DNA and a small genome that encode only a few proteins. They replicate by a rolling circle replication mechanism briefly, by initially converting single-stranded DNA (ssDNA) into double-stranded DNA (dsDNA) intermediates which then serve as templates to amplify the viral dsDNA and to produce mature ssDNA genomes. Genetically engineered virus resistance has been reported in the literature before typically by a pathogen derived resistance (PDR)

[1] Science Daily. Genetically Engineered Maize Is Resistant To Maize Streak Virus.9 July 2007. http://www.sciencedaily.com/releases/2007/07/070708075149.htm

[2] Physorg.com. First all-African produced genetically engineered maize is resistant to maize streak virus. 8 July 2007. http://www.physorg.com/news103082801.html

[3] Ebro, K. (2001) South Africa – CGIAR Partnership Results in New Maize Varieties With 30 to 50 Percent Higher Yields. 21 May. http://www.worldbank.org/html/cgiar/press/news010521.pdf

[4] de Grassi, A. (2003) Genetically Modified Crops and Sustainable Poverty Alleviation in Sub-Saharan Africa. An Assessment of Current Evidence. Third World Network – Africa. June. http://allafrica.com/sustainable/resources/view/00010161.pdf or http://www.gmwatch.org/archive2.asp?arcid=1007

[5] . Kenya Agricultural Research Institute. Maize Streak Virus. An information sheet. http://www.apd.rdg.ac.uk/Agriculture/Research/CropScience/Projects/IntegratedWeed/MSV%20leaflet%20jan2004.pdf.

[6] Martin, D. P, van der Walt, E., Posada, D. &Rybicki, E. P. (2005) The Evolutionary Value of Recombination Is Constrained by Genome Modularity. PLoS Genet 1(4): e51 doi:10.1371/journal.pgen.0010051

strategy which is based on the insertion of resistant genes that are derived from the pathogen (virus) into the host plant.

The widely reported coat protein mediated strategy has not proved very successful for geminiviruses and the UCT/Pannar team have reported engineered resistance by encoding a C-terminal deletion of the replication associated protein (Rep) which initiates the rolling circle replication of geminiviruses.[7] Briefly, a mutated, truncated (C-terminal deletion) form of the Rep protein gene was used to transform maize plants[8] by biolistic transformation. The gene construct consisted of the gene inserted between the maize ubiquitin (Ubi) promoter and the *Agrobacterium tumefaciens* nopaline synthase (Nos) terminator in pAHC17 and co-transformed with the *bar* containing plasmid pAHC25.[8]

Normally, several copies of the Rep protein bind together to form an oligomer, which initiates replication. In the transformed plant, the transgenic protein integrates into the oligomer and inhibits replication. The reported lines have displayed constitutive gene expression and the UCT/Pannar researchers are still in the process of conducting further research in order to achieve a transformed line with single copy integration and are not at a point where there is an identified transgenic line for field trials.

MAIN CONCERNS AND RECOMMENDATIONS

Reporting on the monitoring and characterisation of inserted gene sequences cannot be taken as an assurance that recombinant DNA methods are very precise. Transformation by particle acceleration (biolistic) is associated with multiple fragments and gene re-arrangements.[9,10] These unintended effects might be difficult to detect in the lab. Whilst targeted insertion has been shown to be quite successful in lower organism such as bacteria and viruses, such an outcome has proven more elusive in higher organisms.[10] The precise insertion sites of transgenes can have an impact on the level and consistency of gene expression producing effects that may range from negligible to lethal.[10]

The lack of sophisticated methods for targeted insertion, especially in higher organisms[9] necessitates more rigorous research into possible position effects prior to the granting of any release of transgenic organisms into the environment. Further, if transgenes behave just like naturally occurring genes, then they have the potential to be inherited in the same way and persist indefinitely in cultivated or free-living populations. Any mixing of native and transgenic plants whether by dispersal, improper handling etc., can result in the spread of transgenes. The consequences, both ecological and evolutionary of crop-to-crop gene flow are only now beginning to be investigated in any meaningful way and the possible exposure of non-target organisms, including humans to novel proteins cannot be discounted.[9] The ACB is of

[7] Mangwende, T., Palmer, K. E., Rybicki, E. & Thomson, J. A.. Inhibition of maize streak virus replication in black mexican sweetcorn suspension cells by various maize streak virus-derived genetic constructs. http://www.mcb.uct.ac.za/msv/msv_session_11.htm
[8] Shepherd, D. N., Mangwende, T., Martin. D. P., Bezuidenhout, M., Kloppers, F. J., Carolissen, C. H., Monjane, A. L., Rybicki, E. P. & Thomson, J. A. (2007) Maize streak virus-resistant transgenic maize: a first for Africa. Plant Biotechnology Journal. 5: 000–000. Personal communication
[9] Greenpeace comments on: SNIF for the deliberate release and placing on the EU market of the 1507 maize, C/ES/01/01. http://www.greenpeace.sefiles/200-2399/file_2308.pdf
[10] Snow, G. A., Andow, D. A., Gepts, P., Hallerman, E. M., Power, A., Tiedje, J. M., and Wolfenberger, L. L. (2004) Genetically engineered organisms and the environment: Current status and recommendations. *Ecological Society of America Position Paper.* ESA Public Affairs Office. February 26, 2004. http://ww.esa.org/pao/esaPositions/Papers/geo_position.htm

the opinion that molecular methods must be devised or developed in order to mitigate these unwanted effects.

Horizontal gene transfer (HGT) is the transfer of genetic material between organisms, outside the context of parent to offspring reproduction.[11,12] It is most commonly recognized as infectious transfer.[13] HGT frequencies are now known to be much higher than originally thought. The evolution of antibiotic resistance, for example, is an indicator of the frequency of gene transfer, given that antibiotics have been used in medicine only for about 50 years.[13] The intentional modification of plants could through horizontal gene transfer result in the unintentional modification of other organisms. What the possible impacts of such gene transfer might be is not known. As far as virus resistant crops are concerned, there is always the potential for recombination between viral transgenes and invading viruses. More recent studies have show that such recombination is highly probable.[10] What the effects of this recombination might be cannot be certain.

Allergencity will need to be assessed. One reason for the failure of identification of GM crops as allergenic is related to the fact that no standardised agreed-upon protocols exist for such testing.[11] No test exists that is fully predictive of potential allergenicity.[14] Allergenicity assessments are limited by the fact that amino acid sequences of most allergens remain unknown. Further, several allergens remain undetected and the state of current knowledge on allergens is that there are full length sequences for just 198 major allergens of which 30 are food allergens. Therefore, whilst matches to known allergens are of concern, failure to make a match does not rule out possibility of novel protein being allergenic.[15] Sound scientific method necessitates independent verification of developer results and research resources need to be allocated to such independent study.

The ACB urge UCT/Pannar to ensure that for all risk assessment studies that a multi-disciplinary team be involved including ecologists and evolutionary biologists. We further request that a desk study and as much lab research as is possible be carried out by this multi-disciplinary team to assess potential unwanted effects. If this process raises any flags including the potential for serious unwanted effects that field trials be placed on hold. This, rather than using the field trials as a research mechanism for determining what the risks might be. As much as field trials are controlled, strict containment is often impossible. Where there is a lack of sufficient relevant scientific information and knowledge regarding the extent of potential adverse effects, the Precautionary Principle referenced in the Biosafety Protocol (Cartagena Protocol on Biosafety to the Convention on Biological Diversity) should be triggered. The precautionary principle states that "where there are threats of serious or irreversible

[11] European Communities: Measures Affecting the Approval and Marketing of Biotech Products (DS291, DS292, DS293). (2004) Third Party Submission by Norway.
[12] Heinemann, J. A. (2003) *Bioscience*. 12, 51
[13] Heinemann, J. A. Gene Ecology Guide to: Measuring Horizontal Gene Transfer. Condensed version of paper published in Nature Biotechnology in September 2004. *Personal Communication*
[14] Taylor. S. L. Review of the development of methodology for evaluating the human allergenic potential of novel proteins. http://www.ilsi.org/file/Chapter2Taylor.pdf
[15] Freese, B. (2001) A Critique of the EPA's Decision to Re-Register Bt Crops and an Examination of the Potential Allergenicity of Bt Proteins. Adapted from "Final Comments for Submission to the Environmental Protection Agency Docket No. OOP-00678B Concerning the Revised Risks and Benefits Sections for Bacillus thuringiensis Plant-Pesticides" (submitted to the EPA on September 21, 2001). Friends of the Earth. 9 December.

damage, lack of full scientific certainty shall not be use as a reason for postponing cost-effective measures to prevent environmental degradation".[16]

The ability of ecosystems to develop gradually, the ability to anticipate environmental health effects and very importantly, the establishment of regulatory mechanisms that can effectively, efficiently and credibly manage risks associated with the use of GMOs has not kept apace with the rapid introduction of GMOs. Traditional breeding practices have an established history of safe use dating back several hundred years as opposed to the application of recombinant DNA technology for human use, which is as young as 22 years when genetically modified bacteria-produced insulin was first introduced and even younger for genetically modified plants at ten years.[11] We respectfully submit that the assessments with regard to the transgenic plant include a long-term study comparing traditional and alternative methods of cultivation including organic methods, especially with regard to issues of biosafety.

Often applicants to the National Department of Agriculture wishing to conduct trials of transgenic plants, omit a great deal of information in their dossiers for public consumption under the guise of it being Confidential Business Information. Without basic information relating to the GE events, the public cannot have confidence that adequate safety is being ensured. This, especially in the light of the reports of incidences of contamination from GE trials. ACB urge UCT/Pannar to engage fully with the public and that complete and accurate information be made available to interested parties.

Typically, GE seed is a patented and sold by the developer selling at much higher prices than hybrid seed varieties. Monsanto for example, charges 60% more for Bt maize than the cost of traditional hybrid seed varieties, the so-called "technology fee".[17] Pannar is the largest seed company in South Africa with an international footprint. Thus in regard to its intended support for subsistence farmers, we would like more detail on how, should the transgenic seed be developed to a commercial stage, UCT/Pannar intend marketing this newly developed maize, who the target group would be and what the cost to the farmers would be?

[16] Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Adopted in Montreal on September 11, 2003. http://www.biodiv.org/biosafety/protocol.asp
[17] Kirsten, J. & Gouse, M. The adoption and impact of agricultural biotechnology innovations in South Africa. http://www.up.ac.za/academic/ecoagric/fulltext/2002-25.pdf

ANNEXURE 1

CORRESPONDENCE BETWEEN THE AFRICAN CENTRE FOR BIOSAFETY AND DIONNE SHEPHERD, DEPARTMENT OF MOLECULAR AND CELL BIOLOGY, UNIVERSITY OF CAPE TOWN. (ALL RESPONSES BY DIONNE SHEPHERD IN BOLD)

27.08.2007

I am writing from the African Centre for Biosafety (www.biosafetyafrica.net) and we are keen to know when you plan to submit an application to the Registrar: GMO Act, for permission to conduct field trials with this GM maize.

There is been a great deal of news on this, and thus, we we have been engaged in the biosafety debates in SA for several years now, are understandably very keen to engage with this issue. Please, do give us some indication and also, may we make a plea to you, to kindly ensure that the advertisement for this GM event, be published in the Mail and Guardian, as well as the Business Day, two national newspapers, in order to ensure greater transparency and to elicit greater public participation.

I await your advices on the time lines, for the field trials

28.07.2007

Thanks for your email; I understand your concern for transparency and my colleagues Ed Rybicki, Jennifer Thomson and I will gladly engage with you. The MSV-resistant transgenic lines that we have at the moment all have more than one copy of the transgene (3-4), and although we could apply to perform field trials on these they are most likely not commercialisable due to the multiple integration events. So we are busy trying to get a single copy event at the moment. Once we have that we will perform the risk assessments and apply for contained field trials on that line/s. We will then also advertise the GM event. Meanwhile we are performing tests on the protein itself (e.g. allergenicity; digestibility).

28.07.2007

(a) What do you refer to when they speak of 'transgene'? Are you able to be a bit more specific about this? In particular, can you clarify whether you have several copies of the whole construct or just of parts of it? Can you also indicate whether you have tested for stability of the construct when inherited over generations or outcrossed?

(b) How does the target effect work? Apparently a novel protein is expressed, what is that? Anything known regarding the mode of action of that compound?

(c) Can you also provide measurements of the concentration of the novel protein in various tissues? Can you share with us, what would be your goals for the field release trials?

(d) What are you planning regarding non-target effect testing?

(e) Have you already done any expression trials on the additional transgenes in closed environments? Do you have any data available about the transgene and transgene product expression from the greenhouse experiments? Can you share these with us?

29.08.2007

We'll try to answer your questions as fully as possible by the end of today. I just wanted to clarify though, that the only lines we have such data on are those that will never be released. I imagine you will be more interested in the line/s that we eventually want to do field trials on? (Which, as I said before, we don't even have yet because we are trying to get single copy integration).

30.08.2007

It occurred to me that most of the answers you are looking for can be found in the recent publication on the work, which I've attached.

Briefly, the transgene is derived from the MSV replication associated protein gene (Rep) and is used in a pathogen derived resistance (dominant negative mutant) approach. It is highly truncated and mutated to render it non-functional.

(b) The virus' native Rep protein functions as an oligomer (several Rep molecules bind to each other) in order to replicate the virus. The novel protein produced by the transgenic plant binds to the native virus Rep protein, thereby preventing its function, resulting in inhibition of virus replication.

(c) All we know is that the protein is expressed constitutively. We don't know the concentration in various tissues. The plants we have at the moment are not going to be released (for field trials or otherwise) and so we will perform the bulk of our molecular studies and risk assessments on the lines that we do intend to release for field trials. Briefly, our goals for trial release would be to determine the effectiveness of the resistance strategy in a contained field over a few growing seasons and at the same time to perform all the necessary risk assessment (including an environmental risk assessment).

(d) We will follow the guidelines laid out in the ERA framework. It's worth noting that MSV is present in large quantities naturally in the environment (in maize and grasses) and since the transgene is derived from MSV we don't envisage a problem with non-target organisms; however we will do all the tests necessary.