

Objection to intentional trial release of genetically modified grapevine plants into the environment at Welgevallen, Stellenbosch, submitted by the Institute of Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University

Introduction

Biowatch South Africa is a national non-governmental organisation concerned with issues of biodiversity, biosafety, social justice and food security. We welcome developments in biotechnology which help to advance our economy.

We are objecting to the application from the University of Stellenbosch's Institute for Wine Biotechnology to conduct field trial with transgenic grapevines because:

- There has been an absence of information conveyed in a meaningful way to the public about the issue
- There are significant discrepancies between the public notice and the application to the Registrar Genetic Resources
- The application inadequately addresses biosafety risks and horizontal gene transfer
- The application contradicts environmentally-friendly and sustainable initiatives being spearheaded by some of South Africa's largest wine producers, such as the Biodiversity and Wine Institute.

The basis of our objection is set out in two parts – scientific concerns and other concerns. Biowatch South Africa commissioned an independent scientist to provide an opinion, based on information to which Biowatch South Africa was able to gain access. Biowatch South Africa also conducted a telephonic survey of wine estates to establish whether they were aware of the application. The results are contained in the section headed other concerns.

A. Scientific opinion

By William H.L. Stafford PhD, researcher and lecturer at the Advanced Research Centre for Applied Microbiology, University of the Western Cape.

1. General observations

The aim of the field trial, as stated in the public notice, is “to evaluate the long term stability and expression of introduced transgenes in grapevine under normal field conditions”.

Long-term stability implies stable inheritance (over several generations). But it seems that this study is only addressing stability of a single generation. The term “long-term” needs to be defined and the number of years specified.

The notification states the transgenic cassette uses the uidA and the 35S-CaMV promoter genetic elements in order to assess the stability of transgenic lines. However, it is not entirely clear if there are any other genetic elements (eg. insert DNA) present in this transgenic cassette.

There is also a contradiction between the public notification and more detailed documents, contained in the application to the Registrar Genetically Modified Organisms. The public notification states that the transgenic grapevines contain the cauliflower mosaic virus promoter (35S-CaMV). But the detailed application to the Registrar states that the subterranean clover stunt virus promoter is used (double sc4).

The application also indicates that the transgenic grapevines contain an additional marker gene which is not mentioned in the public notification. These are dealt with in greater detail below.

2. Suitability of 35S-CaMV promoter and uidA B-galacturonidase marker for transgenic grapevine

The viral 35S-CaMV promoter carries certain biosafety risks and many transgenic lines under development use non-viral promoters. It is, therefore, unclear why this construct under development has chosen this promoter.

Briefly, the biosafety risks of the viral 35S-CaMV promoter include:

- Increased recombination (rearrangements, deletions, insertions). There is evidence from the laboratory (Koholi et al. 1999) and field studies (Quist and Chapela 2001, Collonier et al., Ho et al. 2000) that the 35S-CaMV is a recombination ‘hotspot’.
- The 35S-CaMV results in very high expression levels that may result in unintended (pleiotropic) effects from the expressed transgenes.
- Increased recombination with other viral elements and the creation of new viruses (Wintermantel et al. 1996, Vaden and Melcher 1990, Greene et al. 1994).

The uidA marker is also a poor choice for many agronomic crops. It is of particular concern since B-glucuronidase expression in plants increases the number and lifespan of feeding aphid pests (Cherqui et al. 2003), causing grapevine plant damage and consequent yield losses. This also increases the biosafety risks since aphids are hosts to numerous viruses that infect plants thereby increasing the risks of negative unintended effects due to viral recombination (with elements of the transgenic 35S-CaMV cassette) and the creation of new viral pathogens (Greene et al. 1994).

It is also noteworthy that the uidA gene of the transgenic cassette is derived from E.coli which naturally resides in the human intestine. The sequence homology (uidA) of ingested transgenic DNA (grapes) to the uidA of the E.coli resident in the intestine increases the probability of horizontal gene transfer of the transgenic cassette to the genome of intestinal bacteria (de Vries et al. 1998, Netherwood et al. 2004).

Given that the 35S-CaMV is active in E. coli (Asaad and Signer 1990), this may result in abnormally high levels and/or aberrant B-glucuronidase expression in the human intestine. The effects on human health are unclear but high levels of B-glucuronidase in breast milk are thought to contribute to, or cause, bilirubinaemia (jaundice) in infants (Gaffney et al. 1986, La Torre et al. 1999). Effects may also be additive if this transgenic cassette is used for both edible grape (Sultanas) and for wine (Charonnay) since alcohol also effects liver function.

The public notification states that the transgenic grapevine contains the cauliflower mosaic virus promoter (35S-CaMV). However, the application to the Registrar contradict that of the public notification and states that the transgenic contains subterranean clover stunt virus promoter (double sc4) (pages 5 and 34 of the application).

The detailed documents (the application to the Registrar) also indicate that the transgenic grapevine contains an additional marker gene - nptII (neomycin phosphotransferase) for resistance to the B-aminoglycoside antibiotics, neomycin and kanamycin. This is not mentioned in the public notification and carries additional biosafety risks that are particularly relevant to South Africa, since cross resistance of B-aminoglycoside antibiotics is well recognized and spectromycin is used to treat diseases such as TB (Heifets, L. B. 1991, Onaolapo J. (1994) and WHO 1997).

The release of transgenics containing antibiotic resistance genes will spread antibiotic resistance to pathogenic bacteria, thereby compromising the ability to treat diseases. Current biotechnology recommendations are to phase out the use of these markers. Since the uidA B-glucuronidase also serves as a marker, the npt11 is not needed for the study and can be avoided.

3. Suitability of methods

The method used to assess the stability of the transgenic cassette relies upon the expression of the uidA B-glucuronidase marker gene. However, this alone cannot establish the integrity, and hence, stability of the transgenic cassette. For example, any duplications in uidA gene or mutations/truncations/rearrangements in other parts of the transgenic cassette may still allow expression of uidA resulting in the false conclusion that the cassette was stable.

Clearly, DNA molecular methods should be used to establish the stability and integrity of the transgenic cassette. This should include the following:

- PCR of genomic DNA from several plants (population of 20+ individual plants) with primers flanking the genetic elements of the transgenic cassette (i.e. primers flanking 35S-CaMV and uidA). DNA sequencing of selected PCR products should be carried out to determine if single mutations have occurred. Similarly, inverse PCR should be used to determine the flanking regions of the cassette so that stability of the insertion at a defined site in the plant genome can be determined.
- Quantitation of these genetic elements by real-time PCR (or quantitative hybridization by Southern blotting).
- Experiments using comparative genomics are required to fully establish genome stability of transgenic lines. Techniques such as repPCR, RAPD and comparative genome hybridization (CGH) have been shown to be effective in establishing genome similarity (Bao et al. 1993, Pinkel and Albertson 2005). This is required since fragmenting and scattering of the transgenic cassette in the genome (transpositions with rearrangements and deletions) may result in loss of the primer binding sites or a large distance (>10kbp) between genetic elements of the cassette, giving in false negative results by when detection is carried out by standard PCR.

4. Biodiversity inadequacies

It is unclear from the notification if any environmental monitoring or assessment will take place, as required under the National Environmental Management Act of 1998 (NEMA) and the Biosafety Bill (Bill number 1576). There is a general lack of monitoring of the effects on biodiversity (such as soil microbiota) so that the proposed release fails to meet regulations of NEMA.

Although the locality of the trial release and netting will shield the transgenic plants from the wider environment, the method of bagging pollen will not be able to ensure pollen containment.

Furthermore, there may be effects on insects, small enough to pass through the netting, and soil biodiversity, such as, earthworms, non-flying insects or insects that have hatched from eggs in situ, as well the soil microorganisms (Bacteria, Fungi, Archaea) that are vital for soil health and ecosystem functioning. These need to be monitored.

The public notice and the application to the Registrar fail to mention the netting size (eg. Page 38). This is crucial to risk assessment for containment and gene escape and effects on biodiversity.

The monitoring of unintended effects should also include addressing gene escape (horizontal gene transfer) from decomposing grapevine leaf litter to soil microorganisms using appropriate molecular methods (ie. rRNA clone libraries, DGGE microbial profiling, microarrays) (Kowalchuk et al. 2003).

The molecular methods that are proposed to study gene stability and integrity are totally insufficient because the PCR primers (figure 1 page 27) used are not specific for the transgenic cassette (will detect uidA homologs from other species including E. coli and human) and so will be susceptible to random errors (false positives) in PCR detection of transgenics.

This cannot be accounted for by a simple negative control (no DNA). Primers directed to other regions of the cassette should be used to distinguish transgenic and non-transgenic and are also needed to determine transgene integrity (see the detailed objection for recommended molecular methods to determine transgene stability and integrity of the cassette).

It is stated in the application that the level of outcrossing is low (pages 35 and 37) and that the transgenic grapevine is surrounded by non-transgenic grapevine (page 38). However, there is no indication of assessments of gene flow to the non-transgenic vines.

Lack of experimental study to determine that this specific transgenic does not result in significant outcrossing to surrounding plants means that the surrounding plants must also be removed and destroyed as detailed (by incineration). Failure to do so would result in biosafety risks of unknown magnitude which are unacceptable for a trial release.

5. Conclusions

The aims of this field release are to assess the stability of transgenic grapevine containing genetic elements of the 35S-CaMV promoter (viral) and uidA B-glucuronidase (E.coli bacteria).

These genetic elements are a poor choice since the 35S-CaMV carries risks in the generation of new viral pathogens and the expression of B-glucuronidase in plants has been shown to increase the numbers and survival of aphid pests; with obvious negative agricultural effects.

Furthermore, upon human ingestion of transgenic grapes the elevated grape B-glucuronidase could have a direct effect and/or the transgenic cassette could be transferred to natural intestinal E.coli bacteria- both may result in B-glucuronidase over-expression and liver dysfunction (jaundice).

The stated methods used to assess transgene stability will not be able to accurately determine transgenic stability and integrity and more detailed molecular DNA methods are required.

There is no indication of assessments to determine the effects on the interacting environment (the netting used will not keep out small visiting insects) or the immediate environment (soil micro-organisms) in terms of biodiversity and horizontal gene transfer of these transgenes.

In summary, the value of the study is, therefore, questionable since the methods used will not be able to address transgene stability and integrity. The limited data gained from this study in terms of transgenic stability are also questionable since it is suggested that there is no proposal for general release. What are the exact intentions of developing this transgenic cassette?

B. Other concerns

1. Public notification

It is of great concern that the public notice and the application to the Registrar Genetic Resources differs significantly in terms of the promoter which is to be used in the transgenic grapevines.

The public notice also does not mention that the transgenic grapevines will contain an additional marker gene which is antibiotic resistant.

As stated above, in the section on scientific concerns, these facts have an important bearing on biosafety. The omission of these important facts from the public notice limits meaningful and informed public input and somewhat defeats the purpose of the public notice.

The highly technical language in which information in the public notice is couched also does little to encourage the public, even the wine and grape industry, to engage with the merits of the application. And when the technical terms are examined, it is apparent that a crucial aspect of the field trial is not revealed - what specific genetic modification events are being tested and what these are meant to do.

As an exercise in simply meeting a requirement that applications for permission to conduct activities with genetically modified organisms be advertised, the notice cannot be faulted. But the purpose of such a public notification must surely be to get genuine public input. The public notice fails to do this. It is, therefore, not surprising that few wine and grape growers are aware of the application.

2. Lack of public awareness of the application

Biowatch South Africa is aware that the public notice was placed in the following newspapers and media:

- Eikestad Nuus
- Cape Argus
- Die Burger
- The University of Stellenbosch's bulletin
- The website of the Stellenbosch University's Institute for Wine Biotechnology

Nevertheless a significant number of wine estates are not aware of the application. Biowatch South Africa conducted a telephonic survey of wine estates, including South Africa's largest and premier wine producers, distributors and marketers with significant international presences

Of the estates and companies surveyed, 73.68% were not aware of the application.

Of the estates and companies surveyed who were aware of the application, 50% were very concerned about its implications. Said a group manager of one of South Africa's largest wine company: "This is not appropriate to have in South Africa."

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