

Rebuttal to a review of Dona and Arvanitoyannis 2009

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1. Part one

- 35S-Promoter and the stability of transgene inserts
- Antibiotic marker chapter, status 20090725

2. The claim for part one on the 35S promoter and Antibiotic marker genes

Summarized in the abstract published:

“As genetically modified (GM) foods are starting to intrude in our diet concerns have been expressed regarding GM food safety. These concerns as well as the limitations of the procedures followed in the evaluation of their safety are presented. Animal toxicity studies with certain GM foods have shown that they may toxically affect several organs and systems. The review of these studies should not be conducted separately for each GM food, but according to the effects exerted on certain organs it may help us create a better picture of the possible health effects on human beings. The results of most studies with GM foods indicate that they may cause some common toxic effects such as hepatic, pancreatic, renal, or reproductive effects and may alter the hematological, biochemical, and immunologic parameters. However, many years of research with animals and clinical trials are required for this assessment. The use of recombinant GH or its expression in animals should be re-examined since it has been shown that it increases IGF-1 which may promote cancer.”

Dona, A. & Arvanitoyannis, I.S. (2009)

Health Risks of Genetically Modified Foods.

Critical Reviews in Food Science and Nutrition, 49, 2, pp 164 - 175

<http://www.informaworld.com/10.1080/10408390701855993>

AND <http://www.botanischergarten.ch/Food/Dona-Health-Risks-GM-Foods-2009.pdf>

3. Comments by Klaus Ammann and colleagues cited

3.1. General comments

This paper, published recently in “Critical Reviews in Food Science and Nutrition” by the internationally well known editorial house Taylor & Francis, needs to be critically commented for a multitude of reasons:

In a first overview, the reader will find a lot of mostly unconfirmed concerns about the safety of foods derived from GM crops, the citations are extremely filtered in a way to depict a negative picture on GM crops, and the review authors seem to lack proper knowledge about the field of food safety as a whole.

They also publish numerous paragraphs as their own writings, whereas they are just taken by copy-paste from other publications, and worse: those placatory passages are selected from papers with a negative bias and with notorious contents, which have been rebutted recently and for the majority even some years ago. Most of those rebuttals are written by the best authorities in the field, well publicized and easily obtainable in the internet or in libraries from the best peer reviewed journals. Thus Dona et al. give the uninformed reader the wrong picture, as if the food safety situation in 2008 would still be precarious. This is simply not the case and in summary this is a blatant example of scientific distortion of the overall picture in this field of scientific research on food safety.

The text below will give some examples, always supported by peer reviewed literature, which is available in abundance. It is hard to understand that Taylor & Francis let pass such a low quality review with numerous errors, and it is even harder to understand that major efforts in food safety research are simply ignored in this review, or mentioned in a misleading way, such as giving only the outlook and summary comment on one of the major efforts 'ENTRANSFOOD' in the European research on food safety: Citing only (Kuiper et al., 2004), which concentrates on some future research efforts, gives the erroneous picture, that ENTRANSFOOD came to the conclusion that food safety is not yet secured with the GM crops. But if you make the effort of reading the *major official summary* of ENTRANSFOOD (Konig et al., 2004), agreed upon by all researchers participating in this project, then you easily fall on their major conclusion:

"In conclusion, the food safety assessment paradigm as described in this paper, under which any differences in the new food are identified and any hazards and risks characterized, relative to the conventional food or product, clearly establishes whether the test food derived from a GM crop is as safe as the conventional counterpart. It can even be argued that foods from GM crops are better characterized than other non-regulated plant-derived foods, due to the additional rigor in the current regulatory requirements and testing regime compared to that for conventionally-bred crops."

It should be mentioned, that this summary is based on an impressive number of joint research papers which have been carefully coordinated in their conclusions by a consortium of the most renowned researchers in food safety today. But let us start with some concrete examples in order to illustrate one of the harsh negative but unfounded statements of the review:

4. Concerns about the 35S promoter used to enhance the expression of transgenic constructs

Under the treacherous title: ***Potential Effects on Human Health resulting from the use of Viral DNA in Plants p. 167*** the authors set right from the beginning a negative tone:

Based on a publication managed by the same publisher Taylor & Francis (van Ho et al., 2000) the review authors are parroting the notoriously alarmist and often refuted opinion views of Mae van Ho and her colleagues from the ISIS institute, they do not cite the original source (van Ho et al., 1998) and avoid to reveal to the reader, that those papers have been rebutted properly and in detail ever since by some of

the most renowned virologists (Hull et al., 2000a; Morel & Tepfer, 2000) see also the comments of Chris Leaver in (Hodgson, 2000). Here the abstract of (Hull et al., 2000a), which is actually sufficient:

“The 35S promoter, derived from the common plant virus, cauliflower mosaic virus (CaMV), is a component of transgenic constructs in more than 80% of genetically modified (GM) plants. Alarming reports have suggested that the 35S promoter might cause accidental activation of plant genes or endogenous viruses, promote horizontal gene transfer, or might even recombine with mammalian viruses such as HIV, with unexpected consequences. In this article, we discuss the properties of CaMV and the 35S promoter and the potential risks associated with the use of the promoter in GM plants, concluding that any risks are no greater than those encountered in conventional plant breeding.”

In another rebuttal of Ho’s views, (Trewavas & Leaver, 2000), in Nature, it is worthwhile to give the full text here:

How nature itself uses genetic modification

Sir—Mae Wan Ho (Ho, 1999) [see also (van Ho et al., 2000; van Ho et al., 1998)] states that genetic engineering is fundamentally different from conventional plant breeding or wide crosses to produce novel crops; thus she concludes that genetically modified (GM) plants need unique tests on their safety. Any new crop should have its composition of major and minor constituents investigated to establish substantial equivalence, and its novel trait independently tested, as has been the case for the present range of GM plants. However, Ho states that special tests are required because “genetic engineering enables exotic genes... [to be] combined in novel constructs, often with viral promoters to make genes over-express continuously. The constructs are inserted into genomes by transformation techniques that cannot control where the genes go, resulting in a range of unpredictable positional effects and rearrangements” (Van Ho, 1998). But this differs little, if at all, from Ho’s description of normal genome behavior in her book², which states: “Genome organization is infinitely variable [and contains] transposons that can excise and reinsert elements in different locations in the genome” and “up to 20% of some genomes may contain reverse transcripts. These processes destabilize genes and genomes, move genes around, mutate, rearrange, recombine, replicate sequences ...”. What could be more exotic than a mutant protein? Some estimates suggest that the genome of some cereals may contain up to 50% retrotransposons; transposons contain end regions that are hot spots for recombination using transposase. The plant genome contains very large numbers of strong promoters that direct expression as strongly as any viral promoter such as the cauliflower mosaic virus 35S promoter. These promoters are used experimentally; many of them are constitutively and continuously expressed because they control the expression of housekeeping genes. We know the consequences of events via random GM insertion from libraries constructed from T-DNA insertion. However, Ho and other critics of GM technology neglect the extent to which selection is made among many GM transformants, as is the case with conventional plant breeding among siblings. Lethal insertions are self-selecting; potentially innocuous insertions are detected by substantial equivalence.

It is important to recognize that all the food we eat has been (and is) continuously genetically engineered by natural phenomena in ways that do not differ in any fundamental way from the current GM technology. Natural genetic modification of wheat (Peng et al., 1999) and rice, for example, enabled the breeding of dwarf crops, used to feed many millions in the ‘Green Revolution’.

To criticize experimental GM technology while accepting the benefits of natural GM implicit in ‘the fluid genome’ (Van Ho, 1998) hints at a not uncommon attitude that sees synthetic pesticides as morally reprehensible but natural pesticides as good. Is this really any different from notions of Original Sin? (Trewavas & Leaver, 2000)

4.1. The details about the 35S promoter debate

The conclusions in the rebuttal to (van Ho et al., 2000; van Ho et al., 1998) by (Hull et al., 2000a):

“From the arguments above, there is no evidence that the CaMV 35S promoter will increase the risk over those already existing from the breeding and cultivation of conventional crops. . There is no evidence that the 35S promoter, or other retro-element promoters, will have any direct effects, in spite of being consumed in much larger quantities than would be from transgenes in

GM crops. Furthermore, there are compelling arguments to support the view that there would be no more risks arising from potential recombination than there are from existing non-transgenic crops."

The answer of (van Ho et al., 2000) [wrongly labeled in the journal as an "Original Article", although not containing a single set of original data]: summarized by herself:

"The 35S promoter in the virus does not transfer into genomes because pararetroviruses like CaMV, do not need to integrate into host genomes to complete their lifecycle; and the virus replicates in the cytoplasm away from the host genome. Nevertheless, some pararetroviral sequences have been found integrated into plant genomes".

(Hull et al., 2000b) give more balanced details and cannot confirm in his parallel publication the concerns of Ho.

*"Unlike viruses of vertebrates and bacteria, the infection cycle of plant viruses is not known to involve integration; because the caulimoviridae lack features associated with the retrovirus integration phase, they are called pararetroviruses. However, virus sequences are found integrated into plant genomes and, in some cases, have recently been implicated in causing episomal viral infection. The first reports of integration were of multiple direct repeats of partial geminivirus sequence, found in *Nicotiana tabacum* (Bejarano et al., 1996). These sequences included only the origin of replication and the adjacent viral replication protein, transcription was not detectable and there was no associated virus infection.*

*The caulimovirus petunia vein-clearing virus (PVCV) is vertically transmitted and hybridizes to the petunia genome (Noreen et al., 2007; Richert-Pöggeler & Shepherd, 1997). Integrated sequences and virus infection have been linked (Richert-Pöggeler & Shepherd, 1997), although there is no evidence that the entire viral genome integrates, and there are no details of the relationship between integrated and episomal virus sequences. Similarly, caulimovirus-related sequences (tobacco-pararetrovirus-like, TPVL) have been found in the *Nicotiana tabacum* genome but no related episomal virus has been detected (Jakowitsch et al., 1999), however, there are two cases in which the integration of virus sequences is strongly correlated with virus infection: banana streak virus (BSV) and tobacco vein-clearing virus (TVCV)."*

And as Hull rightly so outlined, we always have to make a *baseline comparison* in these risk assessments:

"Furthermore, there are compelling arguments to support the view that there would be no more risks arising from potential recombination than there are from existing non-transgenic crops."

This view has recently been confirmed with modern genomic analysis: (Batista et al., 2008; Shewry et al., 2007): Both teams come to the same conclusion:

"We found that the improvement of a plant variety through the acquisition of a new desired trait, using either mutagenesis or transgenesis, may cause stress and thus lead to an altered expression of untargeted genes. In all of the cases studied, the observed alteration was more extensive in mutagenized than in transgenic plants."

Ho's assumptions based on her years old single-minded view that the promoter DNA should be more instable through transgenesis (her supporting citation is a hard to obtain and certainly outdated textbook reference (Old & Primrose, 1994)) has long ago been countered by peer reviewed publications.

4.2. Concerns about the stability of transgenes

Again, the review authors come forward with unsubstantiated descriptions of transgenesis, which do not reflect the present state of knowledge and experience:

“Moreover, research into epigenetics has also revealed that genes account for only a part of the control of the biochemistry of organisms, and organisms have a level of control above genes that interact with genes explaining why genetic engineering is so unpredictable, with different results produced by each attempt and why the products are often unstable.”

Since the early beginnings of transfer of foreign genes the stability of transgenes and promoters has been subject to intensive studies and experiments. It is an old hat for all molecular plant breeders that they have to test on transgene stability, this starts usually with testing whether the transgenes are situated in one location and thus following the mendelian rules or not. (Gahakwa et al., 2000; Horvath et al., 2001). The abstract may give the answers to the unfounded concerns as one of hundreds of examples:

“The success of contemporary breeding programs involving genetic engineering depends on the stability of transgene expression over many generations. We studied the stability of transgene expression in 40 independent rice plant lines representing 11 diverse cultivated varieties. Each line contained three or four different transgenes delivered by particle bombardment, either by co-transformation or in the form of a co-integrate vector. Approximately 75% of the lines (29/40) demonstrated Mendelian inheritance of all transgenes, suggesting integration at a single locus. We found that levels of transgene expression varied among different lines, but primary transformants showing high-level expression of the gna, gusA, hpt and bar transgenes faithfully transmitted these traits to progeny. Furthermore, we found that cry1Ac and cry2A transgene expression was stably inherited when primary transformants showed moderate or low-level expression. Our results show that six transgenes (three markers and three insect-resistance genes) were stably expressed over four generations of transgenic rice plants. We showed that transgene expression was stable in lines of all the rice genotypes we analyzed. Our data represent a step forward in the transfer of rice genetic engineering technology from model varieties to elite breeding lines grown in different parts of the world.”

4.3. The details:

It is interesting to follow up the scientific debate, beginning in the early 1990ties, where the debate was still not based on solid knowledge about those interrelationships between transgenes and the promoter, both sides were prudent in their conclusions and basically agreed that more molecular data are needed before going into a grand field experiment (Greene & Allison, 1994; Hull, 1994).

In the course of this debate, many studies suggest that recombinant viruses would likely not become established in transgenic plants unless the transgene conferred a significant selective advantage over the wild type virus (Bruening & Falk, 1994a, b; Falk & Bruening, 1994; Gibbs, 1994). In the case of a herbicide tolerance or insecticidal trait under control of the CaMV 35S promoter, it is hard to envision an RNA-based recombination mechanism similar to that described above, as there is no sequence homology between the genes for these traits and any viral genes. And if it were to occur, it is equally hard to imagine how a gene encoding for herbicide tolerance or insecticidal activity would confer any selective advantage to a plant virus . (Powell, 1999)

Numerous carefully carried out experimental studies (Blanc et al., 1993; Papparini & Romano-Spica, 2006; Sanders et al., 1987) ever since provided a lot of reassuring data that the concerns of van Ho in the development and regulation of transgenic crops are not valid. In a major long term study on transgenic trees (Maghuly et al., 2007) recently conclude:

“Overall, these results suggest that transgene expression in perennial species, such as fruit trees, remains stable in time and space, over extended periods and in different organs, confirming the value of PAR as model species to study season-dependent regulation in mature stone fruit tissues.”

This is as early as 1994 after a very thoughtful review of the available virological literature at that time the clear conclusion of (Falk & Bruening, 1994):

“We believe that it is unlikely that recombinants between transgene RNA and viral genomic RNA will occur at frequencies greater than they already are occurring by recombinations between virus genomicRNAs in natural conventional and subliminal infections. It also is unlikely that any given new virus will be more viable than competing viruses throughout the full infection cycle: transmission to the new host, un-coating and gene expression, replication, assembly of new virions, and possibly infection of alternative hosts. “

“The virus-resistant cultivars developed by traditional plant breeding have fostered the emergence of virulent virus strains (Dawson & Hilf, 1992) but the cost to agriculture of such virus strains is much less than the cost of abandoning plant breeding. Similarly, the potential benefits of engineered resistance genes far outweigh the vanishingly small risk of creating new and harmful viruses in significant excess over those being created by natural processes.”

The subsequent debate between Bruening, Gibbs, Hull and Mellon do not bring new arguments related to the risk statements of Bruening cited above: (Bruening & Falk, 1994a, b; Gibbs, 1994; Hull, 1994)

In a personal communication 23. March 2009 Hull refers to the fact, that rubisco as the most widespread enzyme should, if Ho's view would match reality, interact much more often and cause a lot of genetic exchange with the CAM retrovirus, since it has much more genomic similarities, but it obviously does not so.

It is astounding, that Ho got away with a superficial response to the rebuttals (van Ho et al., 2000) in the same and other journals. According to the Taylor & Francis' own principles Ho's text should have been subject to serious editorial debate, even possible retraction, see http://www.informaworld.com/smpp/authors_journals_corrections~db=all . For clarity, the paragraph on retraction is cited here in full extent:

“Retractions

A retraction is a notification of invalid results. Retractions are judged according to whether the main conclusion of the paper is seriously undermined as a result, for example, of subsequent information coming to light of which the authors were not aware at the time of publication. In the case of experimental papers, this can include e.g. further experiments by the authors or by others which do not confirm the main experimental conclusion of the original publication. Readers wishing to draw the editors' attention to published work requiring retraction should first contact the author of the original paper and then write to the journal, including copies of the correspondence with the author (whether or not the correspondence has been answered).”

Further information about the policy of Taylor & Francis see <http://www.tandf.co.uk/journals/ethics.asp>

This is such an obvious case of negligence of citing appropriate papers which do not fit your own opinion, so that right from the beginning one doubts about the neutrality of the review authors – to be explicit, both author teams cited here are targeted with this remark, Ho et al. and Dona et al.

As an experienced university teacher as David Tribe, having for many years actively researched the safety of genetically modified foods, he is outraged by reading again the old views of Mae van Hoe rehashed in an uncritical way, he cites:

This superficial review of the debate around the 35S promoter has also been correctly summarized by (Hodgson, 2000) with statements which should have once and for ever settled the case, citing for instance C. Leaver, who consulted many well known virologists and came to the following verdict:

“Having consulted a number of the UK’s leading plant virologists, Chris Leaver from the University of Oxford rejected the suggestion made by Ho et al. that CaMV 35S could be transferred from plants to green algae, yeast, and Escherichia coli on similar grounds. He noted that such a transfer had not occurred even though, throughout history, humans had consumed huge quantities of the CaMV 35S promoter by “eating our greens.” He also rejected as “pure fiction, and lies” the suggestion that CaMV might reactivate dormant hepatitis B virus or create new viruses because there was a phylogenetic relationship between CaMV and human hepadnaviruses (such as hepatitis B). Leaver points out the obvious defect in this argument: the two viruses never replicate in the same cells; CaMV replicates in plants and hepatitis B in animals.”

This is indeed not a good beginning for the critical reader of the review of (Dona & Arvanitoyannis, 2009) when you have to realize, that major critiques of Mae van Ho’s papers are simply ignored and on top of this the sharpest rebuttal in Nature Biotechnology is also totally ignored in its contents, although the review authors had the audacity to cite it, but did not make any comments.

5. Concerns about the use of antibiotic marker genes for selection purposes

Then it goes on in the same superficial style:

“An area of concern focuses on the possibility that antibiotic resistance genes used as markers in transgenic crops may be horizontally transferred to pathogenic gut bacteria, thereby reducing the effectiveness of antimicrobial therapy”.

This is another of the numerous fear monger sentence which ignores again a bulk of scientific literature which demonstrates that the risk is negligible. In a cheap way the authors allude to the risk of using antibiotic marker genes for the selection of transgenic cells, avoiding to cite a substantial body of literature giving data stating the exact contrary: namely that the use of those marker genes is benign and has not caused the slightest detrimental effect. Instead they cite a relatively outdated papers summing up clearly outdated facts on possible horizontal gene transfer. The probability of a horizontal gene transfer is very low, and in the soil there are masses of bacteria with the same antibiotic resistance genes.

To represent the today’s state of knowledge, it is sufficient to cite the abstract of one of the major meta studies taking into account many datasets and reviews (Ramessar et al., 2007) (it seems that the review authors do not know this major study, or worse: They ignore it, because it does not fit into their negativistic concept.

“Selectable marker gene systems are vital for the development of transgenic crops. Since the creation of the first transgenic plants in the early 1980s and their subsequent commercialization worldwide over almost an entire decade, antibiotic and herbicide resistance selectable marker gene systems have been an integral feature of plant genetic modification. Without them, creating transgenic crops is not feasible on purely economic and practical terms. These systems allow the relatively straightforward identification and selection of plants that have stably incorporated not only the marker genes but also genes of interest, for example herbicide tolerance and pest resistance. Bacterial antibiotic resistance genes are also crucial in molecular

biology manipulations in the laboratory. An unprecedented debate has accompanied the development and commercialization of transgenic crops. Divergent policies and their implementation in the European Union on one hand and the rest of the world on the other (industrialized and developing countries alike), have resulted in disputes with serious consequences on agricultural policy, world trade and food security. A lot of research effort has been directed towards the development of marker-free transformation or systems to remove selectable markers. Such research has been in a large part motivated by perceived problems with antibiotic resistance selectable markers; however, it is not justified from a safety point of view. The aim of this review is to discuss in some detail the currently available scientific evidence that overwhelmingly argues for the safety of these marker gene systems. **Our conclusion, supported by numerous studies, most of which are commissioned by some of the very parties that have taken a position against the use of antibiotic selectable marker gene systems, is that there is no scientific basis to argue against the use and presence of selectable marker genes as a class in transgenic plants.**"

It is also important to cite a paper published one year after the meta study above: (Demaneche et al., 2008), the conclusions are exactly the same (and again ignored by the reviewers)

"The successful transfer of transgene-borne antibiotic resistance genes to bacteria might be unavoidable according to a plethora of scientific data. This includes the long-term DNA persistence in soil, the heterogeneous soil structure favoring contact between DNA and bacteria, the prokaryotic origin of the plant transgene sequences that represent a specific risk for a facilitated integration in a bacterial genome by HGT as demonstrated under laboratory (41), and greenhouse conditions (39). In addition, in the Bt176 event that we investigated here, the bacterial promoter was introduced concomitantly with the antibiotic resistance gene that would facilitate its expression in a potential recipient. Finally, these GMPs were cultured in the same field for 10 successive years, making this GMP-field combination particularly suitable to address gene transfer questions and impact on soil bacteria.

However, the detection of such events remains very difficult, and, in this study, we, like others before, did not detect any cellular or molecular evidence that the blaTEM116 gene from the Bt176 transgenic plant was transferred to bacteria. If such transfer events ever happened (although undetectable), they apparently remain without consequences on the soil bacterial community structure. In addition, the use of the sensitive microarray-based hybridization technique failed to detect any significant changes in soil bacteria that could be specifically related to the presence of the transgenic plants or to the expression of the transgene, including cry genes. These results are probably partly due to the low frequency at which these transfer events happen and at a limited efficiency of the investigation protocols. However, they are also and mainly because these genes are already present in soil. Bacteria that would have acquired a blaTEM116 gene from the plant would not have a specific selective advantage relative to other resistant bacteria. Our results indicate that indigenous bacteria are already involved in evolutionary processes by point mutations and HGT as evidenced by the polymorphism of the bla gene in the various soils tested. This could indicate that soil is certainly a reservoir in which all bacteria, including clinical pathogens, can acquire the genetic determinants that could permit them to adapt rapidly to present and future antibiotics. These results confirm the interest of considering the evolutionary potential of bacteria when evaluating the impact of GMPs. Our data are sufficiently informative to conclude that the risk that antibiotic-resistant genes in GMPs can pose to commensal and clinical bacteria should be considered as almost null. This risk has to be neglected not because these genes cannot be transferred but because the plethora of genes already present in soil bacteria and the constant evolution to which they are subjected limit the impact that a newly acquired, yet identical, gene from a plant can have."

However, in the phytosphere, here the surface of leaves of transgenic tobacco, there is a possibility of horizontal transgene-transfer demonstrated by (Pontirollo et al., 2009), see the abstract:

"Plant surfaces, colonized by numerous and diverse bacterial species, are often considered hot spots for horizontal gene transfer (HGT) between plants and bacteria. Plant DNA released during the degradation of plant tissues can persist and remain biologically active for significant periods of time, suggesting that soil or plant-associated bacteria could be in direct contact with plant DNA. In addition, nutrients released during the decaying process may provide a copiotrophic environment conducive for opportunistic microbial growth. Using Acinetobacter baylyi strain BD413 and transplastomic tobacco plants harboring the aadA gene as models, the objective of this study was to determine whether specific niches could be shown to foster bacterial growth on intact or decaying plant tissues, to develop a competence state, and to possibly acquire exogenous plant DNA by natural

transformation. Visualization of HGT in situ was performed using A. baylyi strain BD413 (rbcl-Delta PaadA::gfp) carrying a promoterless aadA::gfp fusion. Both antibiotic resistance and green fluorescence phenotypes were restored in recombinant bacterial cells after homologous recombination with transgenic plant DNA. Opportunistic growth occurred on decaying plant tissues, and a significant proportion of the bacteria developed a competence state. Quantification of transformants clearly supported the idea that the phytosphere constitutes a hot spot for HGT between plants and bacteria. The nondisruptive approach used to visualize transformants in situ provides new insights into environmental factors influencing HGT for plant tissues.” (Pontiroli et al., 2009)

This does not mean that the bacteria performing HGT in the phytosphere would be able to build up significant populations in real soil environments, see also (Rizzi et al., 2008).

(Pontiroli et al., 2007) are presenting a balanced view, *including* true baseline comparisons, but also fail to present concrete examples of horizontal gene transfer from higher plants to soil microorganisms, but still offer scenarios to consider:

“The changes in microbial communities associated with growing transgenic crops are relatively variable and transient, in comparison with some other well-accepted agricultural practices such as crop rotation, tillage, herbicide usage and irrigation. Since minor alterations in the diversity of the microbial community might affect soil health and ecosystem functioning, the impact that a plant variety may have on the dynamics of rhizosphere microbial populations, and hence, plant growth and health, as well as ecosystem sustainability requires further study.”

5.1. The details:

There are many major scientific reviews which document the contrary of the alleged statements of Dona et al., either the review authors do not know them or they simply did not care searching for more references, again an extremely sloppy behavior of review authors claiming high scientific standard. Some important citations demonstrated – all papers based on data investigating the concerns in lab and field experiments, whether antibiotic marker genes could have any kind of detrimental effect on soil microbiology or within human and animal digestion systems. None of the more recent papers could demonstrate some negative effects, although some earlier publications did not rule out the *possibility* of horizontal marker gene transfer from the transgenic plant to microorganisms for various reasons and called for more research.

(Badosa et al., 2004; Bennett et al., 2004; Chambers et al., 2002; Gay, 2002; Gerzabek et al., 2004; Goldstein et al., 2005; Halford & Shewry, 2000; Jelenic, 2003; Kim et al., 2004; Montesinos et al., 2002; Netherwood et al., 1999; Nielsen et al., 1998; Nielsen et al., 1997; Ramessar et al., 2007; Redenbaugh et al., 1995; Salyers, 2001; Seveno et al., 2002; Smalla et al., 1993a; Smalla & Sobczyk, 2002; Smalla et al., 1993b; van den Eede et al., 2004; Wendtpothoff et al., 1994; Widmer et al., 1996).

Numerous papers exist, which call for the elimination of antibiotic marker genes proposing various methods, either with marker free transgenesis or with other markers without antibiotic characteristics, and interestingly enough, all do propose this with the motivation of regulatory politics or public concern, not with scientific data underlying *any* negative effects of antibiotic marker genes.

(Cuellar et al., 2006; Daniell et al., 2001; de Vetten et al., 2003; Dufourmantel et al., 2007; Ebinuma et al., 1997; Goldstein et al., 2005; Halford & Shewry, 2000; Hentges et al., 2005; lamtham & Day, 2000;

Jelenic, 2003; Joersbo, 2001; Joersbo et al., 2003; Khan & Maliga, 1999; Kim et al., 2004; Klaus et al., 2004; Lu et al., 2001; Malik & Saroha, 1999; Nielsen et al., 2000; Phipps & Beever, 2000; Pretty, 2001; Rosellini & Veronesi, 2007; Sarin et al., 2006).

An interesting controversy about the topic of horizontal gene transfer between seemingly closely related soil bacteria, one frequently used as a donor of the wide spread protoxin-transgene to create pest resistance in many widespread GM crops, conducted in Nature Biotechnology 2004-2005, ended with a corrigendum of (Heinemann & Traavik, 2005), where they retract the incorrect allusion, that *Bacillus thuringiensis* (the 'donor' of the Bt gene) is so closely related to *Bacillus anthracis*, that potential horizontal gene transfer between the two 'species' could have fatal outcome. (Davison, 2004; Heinemann & Traavik, 2004a, b; Nielsen, 2003; Nielsen & Townsend, 2004a; Nielsen & Townsend, 2004b). One wonders, why the authors did not mention this case – most probably because the rebuttals were published in the same peer reviewed journal.

But let's come back to some more unsubstantiated allegations of Dona et al. (it is not possible to mention them all!)

Dona et al:

"These limitations in the detection of GM DNA should make us reconsider the view that gene transfer cannot occur, which falls in agreement with the findings of (Netherwood et al., 1999; Netherwood et al., 2004) that transgene from GM soya survived passage through the small bowel in human ileostomists"

Then the authors seriously come back to some really old hats in regulation, namely that transgenes have been found to horizontally wander into the gut bacteria of humans, but this under extremely special circumstances, as (Netherwood et al., 1999; Netherwood et al., 2004) have given the full details:

"The inclusion of genetically modified (GM) plants in the human diet has raised concerns about the possible transfer of transgenes from GM plants to intestinal micro flora and enterocytes. The persistence in the human gut of DNA from dietary GM plants is unknown. Here we study the survival of the transgene epsps from GM soya in the small intestine of human ileostomists (i.e., individuals in which the terminal ileum is resected and digesta are diverted from the body via a stoma to a colostomy bag). The transgene did not survive passage through the intact gastrointestinal tract of human subjects fed GM soya. Three of seven ileostomists showed evidence of low frequency gene transfer from GM soya to the microflora of the small bowel before their involvement in these experiments. As this low level of EPSPS in the intestinal microflora did not increase after consumption of the meal containing GM soya, we conclude that the gene transfer did not occur during the feeding experiment."

In this abstract it is clearly stated that in normal gastrointestinal passage the transgenes could not be detected anymore. But some malevolent NGO representatives misinterpreted those results found in the external bowls of the few poor ileostomists (having lost their normal gastrointestinal digestion ability) where indeed some transfer of EPSPS transgenes were detected, but they do not mention this important detail and generalize to make things really worse. The same 'mistake' is now made by the reviewers, a scandal in a scientific journal per se. Did they read too many Greenpeace leaflets ?

Again we encounter a situation in the Dona review, where a huge body of scientific knowledge on antibiotic marker genes is ignored and one begins to doubt whether this is due to sheer ignorance or due to a strongly bioased view of the matter, ignoring on purpose papers not fitting the view of the review authors. And in an unexplainable irony the authors touch on the jelly fish gene as a marker gene,

long ruled out and only used for tracing experiments in order to obtain real time transformation data. (Stewart & McLean, 2008)

6. Concerns about toxic effects of GM food

With all the repeated hints about potential horizontal gene transfer including technical details the uneducated reader gets the impression, that the Bt or Roundup Ready tolerance transgenes – if detected by whatsoever sensitive methods - could do some harm when ingested, which is not the case: (It should be clearly said that those transgenes used in the widespread and regulated GM crops are harmless, they have been tested in numerous experiments and deemed safe. The review fails to cite most of them, and if it does so, it gives an out-righteous false interpretation like in the case of the rat experiments of (Malatesta et al., 2003; Malatesta et al., 2002a; Malatesta et al., 2002b; Malatesta et al., 2005). Dona et al. fail to report about the subtle and open minded debate of the last publication 2005 of the group, where they leave open that the measured organ changes might be caused by other factors than the transgenes involved (the variation of the amounts of phytoestrogens taken up with the soybeans e.g.):

Another example of bias in reporting on scientific results is the mention of the feeding study on trouts by (Ostaszewska et al., 2005), which is mentioned indirectly as a study with GM soybeans. If the authors would have taken the trouble to read the paper in full text, they could have seen that the **study did not work with GM soybeans**, the aim was quite a different one, the results are really interesting: From the abstract:

“The liver cells showed regular development in both species fed the control diet and in pacu fed SBM and SPC diets. On the contrary, the hepatocytes of SBM and SPC-based diet fed rainbow trout showed anomalies. In both species, the average hepatocyte nuclear volumes significantly differed among the feeding groups. The results of histological analyses indicated that absorption and transport of nutrients to liver and pancreas were affected by the presence of soybean products in experimental diets. The SBM diet was beneficial for pacu but adversely affected rainbow trout, while the SPC diet resulted in extensive pathologies of digestive tract and most likely affected nutrient utilization in both species.”

This is an excellent example that you can show organ effects with simply change the protein diet, actually an important argument against the mainstream opinion of this review.

One of the biggest gaffes in the review is the appreciation of Ermakova’s results, which have been thoroughly rebutted in a series of contributions in Nature Biotechnology (Marshall et al., 2007), the experiments have been criticized by a group of food specialists as deeply flawed and scientifically useless. A full account can also be found soon on the website of the Public Research and Regulation Initiative, as for now its given on the following link:

<http://www.botanischergarten.ch/AF-4-Ermakova/AF-4-Ermakova-20090801-opensource.pdf>

Numerous additional critical remarks should be made about this review, but it’s a matter of space and time to write more.

Final remarks: It seems to the author unfathomable, that a renowned editorial house like Taylor & Francis let the paper slip through peer review, since nearly every paragraph contains obvious and blatant mistakes, which are easily detected by experts in the field, and more: it is a clear cut case of filtered citation.

7. Literature cited, including full text links

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