Sálim Ali Foundation
Kanimangalam Post; Thrissur, India
www.salimalifoundation.org
vijayanvadayil@gmail.com

Dr. V. S. Vijayan
Honorary Chairman

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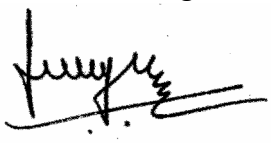
To all Members of the GEAC

Dear Madam/Sir

Kindly find our comments annexed along with this letter on the GM mustard – herbicide tolerant issue. We do hope you will realize the dangers behind it and will take a prudent decision.

With warm regards



V. S. Vijayan
Honorary Chairman, Sálim Ali Foundation
Former Chairman, Kerala State Biodiversity Board
Founder Director, SACON (Sálim Ali Centre for Ornithology and Natural History, a centre of excellence of the MoEF & CC, Govt. of India)

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GM Mustard – an Herbicide Tolerant crop

Dr. V. S. Vijayan
Chairman
Sálim Ali Foundation

The Sálim Ali Foundation has been closely observing the debate over the introduction of Genetically Modified crops in the country and has been keeping the MoEF & CC posted of our comments. In the present case of GM mustard we are particularly worried as it clearly is an herbicide tolerant one and hence will lead to irrecoverable damage to the biodiversity of the country, conservation of which is one of our missions.

We have gone through critically most of the relevant articles and reports and our comments are placed below for the consideration of honourable members of the GEAC.

1. In the case of GM mustard it assumes greater significance unlike the Bt cotton or the Bt brinjal (the latter was not allowed to introduce), because it is a clear herbicide tolerant one that if introduced would devastate the biodiversity of the area. It is still worse that the MoEF & CC is trying to cover up this fact and making deliberate efforts to mislead the public by refusing to classify this as herbicide tolerant (HT) mustard.

2. Considering the over-enthusiasm of the Government of India in introducing GM crops, it is clear that taking such a stand is easier to introduce the first GM food crop in Indian agriculture and to overcome the regulatory hurdles. One should also realize that such a stand also facilitates the easier entry of many HT crops that are immediately in the regulatory pipeline following GM mustard. Other reasons for the government to desist from classifying/ referring to GM mustard as a HT crop appeared to be:

    i) GM mustard lines (parental lines and DMH-11, the GM mustard hybrid) have not been tested as an HT crop. Total denial that it is a HT crop suits the regulators who have already given a safety clean chit to GM mustard.
    ii) Moreover, no protocol has been developed or exists till date to test HT mustard in the Indian regulatory system.
    iii) More importantly, at least two committees[1,2] comprising outstanding scientists recommended against the use of HT crops in India.
    iv) Again, with the evidence[3] of global experience of HT crops in the last two decades, there is an increasing concern over HT crops as it evidently is an undesirable technology.
    v) Therefore, those interested in GM mustard would naturally not be interested in projecting GM mustard as an HT crop for the apparent reason mentioned above.
    vi) It may also be noted that since the next set of crops waiting in the Indian regulatory pipeline for approval for commercial cultivation are also herbicide tolerant, any

rejection of GM mustard on the grounds that it is HT will affect the chances of giving clearance to them also.

3. The government's strong determination in proceeding with a scientific deception of not accepting GM mustard as HT Mustard is evident from its recent response to an interlocutory application in an ongoing PIL on GMOs in the Supreme Court (WP260/2005), where the Government of India says, *"…The petitioner has chosen to refer to DMH-11 as HT DMH-11. The term would be correct only when the herbicide tolerance is the commercial trait. Here it is a selection marker for hybrid seed production …..and not 'Herbicide Tolerance' which globally acronym for….while the presence of Bar gene makes the crop resistant to herbicide 'Basta'* (Phosphinothricin, also known as PPT) - *there is no proposal to use this herbicide in the farmers' field*."
4. It also alleged by the Government that the petitioner was trying to confuse the Court by using the term HT DMH-11. The Government argues that HT gene is used only as a selection marker, but it conveniently forgets that functionally it protects the crop from herbicide. Although it is used as selection marker at laboratory level, it also helps filtering out (un)necessary female plants for hybrid seed production level and, it is the very same gene BAR, which does make the whole crop herbicide tolerant.
5. Arguing against terming / referring GM mustard as "HT mustard" by emphasizing that herbicide tolerance is not meant as a "commercial trait" or that there is "no proposal to use (herbicide) in the farmers' field" or that herbicide use is not recommended, or even including in the Assessment of Food and Environment Safety (AFES) document[4] a line that says "***Additional measures should be taken not to include any chemicals for weed control in the package of practices***" (Page 112) is a proof of treachery misleading the court / reader deliberately.
6. It is to be noted that in India, there is no control over farm level use of any pesticide and farmers never wait for any "proposal" to spray pesticide.
7. Not making a "proposal" to use herbicide does not mean that the crop is HT or not. A classic illustration is that the end use is not regulated and that the current set of GM crop developers have been using glufosinate in their GM mustard R&D and seed production, even though the chemical has not been registered for use on mustard crop by the pesticide regulators! This is also a classic example of how researchers are breaking the rules and regulators are turning blind eye to it.
8. Another point illustrating that, farmers will not abide by recommendations made or not made, and that regulators are incapable of regulating farmer-level usage of herbicide tolerance trait is that the ***illegal herbicide tolerant cotton is being cultivated for many years on thousands of acres.*** That too with the full knowledge of GEAC which discussed the matter but could not make any effective intervention to stop such illegal cultivation. In this context, what guarantee is there that the same will not be repeated in the present case of herbicide tolerant transgenic mustard?

Herbicide Tolerance of GM mustard

9. A description of glufosinate-tolerant crop from an industry sponsored website[5] is quoted below: *"Glufosinate herbicides contain the active ingredient phosphinothricin, which kills plants by blocking the enzyme responsible for nitrogen metabolism and for detoxifying ammonia, a by-product of plant metabolism. Crops modified to tolerate glufosinate contain a bacterial gene* (BAR in our case) *that produces an enzyme that detoxifies phosphinothricin and prevents it from doing damage".*

10. Scientifically, the very expression of BAR gene as described above is exploited at three levels in three ways:

    I. **At the research level, to ensure functional insertion of a novel gene in the laboratory (*in vitro selection*):** "Barnase-Bar" as well as "Barstar- Bar" constructs are used to check whether the construct in the plant cell is inserted well and is working or not. Plants containing Barnase–Bar gene (introduced in female parent) become male sterile and a plant containing Barstar-Bar gene (introduced in male parent) restores male fertility. In the laboratory glufosinate is sprayed on the transgenic plants and the surviving plants are proof of success of the intended insertion. At this level the expression of bar gene is exploited as *in vitro* Selectable Marker Gene (SMG).

    II. **At the seed production level to filter male sterile plants in open fields (*in vivo selection*):** To produce hybrid seed, female plants not containing barnase gene are male fertile and they are required to be killed, especially given the way the female parental line is maintained through back-crossing as in the case of Varuna barnase in the current instance of GM mustard. As the bar gene is attached with barnase, spraying of glufosinate ensures that only male sterile female plants survive. This then prevents self-pollination, forcing the female plant to get fertilized only by outcrossed pollen from male parent, which leads to intended hybrid seed production. At this level, the expression of bar gene is exploited by a seed producer as *in vivo* SMG to kill unwanted female plants.

    III. **At the commercial cultivation level on farmers' field:** The ability of bar gene containing plant to tolerate spray(s) of glufosinate can be used to kill unwanted plants termed as "weeds" in the standing herbicide tolerant crop. Literature[6] on bar, barnase, barstar states, *"The presence of the Bar gene also allows for effective post emergent weed control with the glufosinate ammonium-containing herbicide".* At this level the expression of bar gene is exploited by a farmer to get rid of "weeds".

11. The Bar-Barnase-Barstar technology used in developing the GM mustard is a **package deal** and once it is used at upper level, the character of herbicide tolerance will percolate to the succeeding lower level as the gene is inherited in the next generation and there is no mechanism to deactivate or remove functional bar gene in the F1 plant, which is the DMH-11 hybrid in the case of GM mustard. Purpose of insertion of Bar gene is now clear beyond any doubt irrespective of the term GM mustard as HT crop or not. What matters is the presence of the functional bar gene which confers tolerance to herbicide

12. It is reported[7] that "*most SMGs* (Selectable Marker Genes - like BAR) *express protein products that confer antibiotic- or herbicide resistance traits, and typically reside in the end product of genetically modified (GM) plants. The presence of these genes in GM plants, and subsequently in food, feed and the environment, are of concern and subject to special government regulation in many countries… In recent years, several strategies have been developed to remove SMGs from GM products while retaining the transgenes of interest".* Such Strategies do not make the crop herbicide tolerant; but they have not been employed to develop this GM mustard. Use of BAR genes as markers brings in their own risks.

Evidence of the developer intended to produce a HT crop

13. There is indisputable evidence to prove that the developer used the herbicide tolerance (HT) trait not only as selection marker, but also for potential commercial use to harness the herbicide tolerance trait to curb weeds in the standing crop.

14. The first research paper[8] reporting development of GM mustard by the current set of crop developer cited below describes how its herbicide tolerance ability can be useful in commercial crop cultivation.

15. Dr. Pental and his team published their first research article[8] on glufosinate (Phosphinothricin, also known as PPT) tolerant mustard way back in 2000. It reads, "*We report in this study development of transgenic mustard (Brassica juncea) plants that are resistant to herbicide phosphinothricin (PPT)…herbicides like glyphosate and PPT which are highly effective at low dosage, safe for animals and rapidly degrade in soil could be* ***more useful for weed control. However, these herbicides are non-selective and therefore have to be used in conjunction with transgenic crops that are resistant to these herbicides."***

16. Use of herbicide was advocated in the same article, *"In India, more benefit of herbicides could be in no-till agriculture for moisture conservation in the rain-fed areas and for multiple cropping in the irrigated intensively cultivated areas".* It is a matter for another debate (based on many evidences[9,10,11]) whether Pental and his team were right in declaring the herbicides as highly effective and safe. However, what this paper clearly demonstrates is that Pental and his team intended to introduce mustard cultivar as Herbicide Tolerant (HT) crop both in rain-fed as well as irrigated areas. It is important to note that glufosinate is marketed as Basta in India and patented by Bayer, a German agro-chemical giant (which recently bought Monsanto and further consolidated its hold over the global seed and chemical market).

17. It may be noted that **Pental and his team has a consistent interest in developing herbicide Tolerant Crops,** as they had developed Herbicide Tolerant line for two other herbicides about a decade ago.
18. A paper[12] of Pental et.al. in 2007 in *Current Science* claimed to have developed another herbicide tolerant mustard line to provide "*a viable alternative to* ***phosphinothricin / glufosinate*** *herbicide used in the first generation hybrid using the barnase-barstar system*". This HT transgenic line contains ALS^{dm} (acetolactate synthase) conferring resistance to another herbicide **imidazolinone** - imazethapyr, the active ingredient of "Pursuit", marketed by BASF, another agro-chemical giant. As claimed in this paper, it is a cheaper herbicide compared to Basta. Pursuit is used in soybean crop in Madhya Pradesh. ALS gene is sourced from *Arabidopsis thaliana*, a weedy flowering plant native to Eurasia. This gene, also resistant to one more group of herbicides **sulfonylureas,** which was discovered and patented by DuPont.
19. The Centre for Genetic Manipulation of Crop Plants (CGMCP) of Delhi University, in the light of the two papers mentioned above, seems to have developed several years ago (by 2007) herbicide tolerant GM mustard to tolerate three herbicides, all of them are largely marketed in India by multinational agro-chemical companies.

Glyphosate tolerant mustard is under development

20. Pental and his group at the CGMCP continue their pursuits in genetic modification for Herbicide Tolerance. This time it is Glyphosate tolerant mustard. They have recently approached[13] DBT for grants to *"develop transgenics for resistance to herbicide glyphosate for low-till agriculture and control of root parasite Orobanche,…* ***use of glyphosate to control the weed will require development of transgenic mustard that is resistant to the herbicide".*** This proposal describes two strategies adopted to develop **glyphosate** resistant HT mustard crop. And, it is interesting to note a line in Annexure 9 of this proposal: ***"the most effective strategy among all these*** (it refers to various methods listed earlier) ***is the use of genetically engineered glyphosate-resistant crops"****.* Does it mean anything other than the total commitment of the Pental group - the developer of HT mustard – for genetic modification for herbicide tolerance? It is a paradox that the Government of India still tries to cover this up and say that it is only a selection marker! And, not a herbicide (herbicide tolerant crop).
21. In this context it is worth noting that use of glyphosate is being restricted in many countries[14] including Srilanka (where it is banned), while our researchers are advocating and introducing cultivars which eventually will increase its use. Unfortunately, the government is not only defending but also allowing the backdoor entry of HT crops and funding such research using taxpayers' funds. According to sources, the Department for Biotechnology (DBT) has sanctioned at least a part of the funds requested by CGMCP, for R&D for such risky technologies.

Use of double enhancer promoter to ensure 10 fold expression of HT trait

22. Another very important proof to term GM mustard as HT crop is the **use of double enhancer promoter** in the Barstar gene construct of the EH-2 line (male parent). The AFES document[4] submitted by the sub-committee of GEAC reads, *"In the barstar gene construct, the bar gene, used as a plant selectable marker, is controlled by CaMV35S double enhancer promoter (with duplication of the enhancer region from -90 to -343). This promoter confers a 10-fold increase in the expression levels over the corresponding single enhancer counterpart".* The Barstar gene construct will be naturally inherited by the succeeding F1 generation, called DMH-11, which exhibits high levels of herbicide tolerance with two bar genes, one driven by a single enhancer and the other by a double enhancer. Double enhancer promoter is neither necessary for male parent nor for DMH-11. This itself is a dead giveaway of the crop developer to develop a HT crop. Unless there is an intention to develop HT crop, why was the double enhancer promoter deployed?

Conclusion:

1. GM mustard developed by the CGMCP by all means is a herbicide tolerant (HT) crop. It would be a misnomer to call it by any term other than Herbicide Tolerant crop.
2. There is enough evidence in the documents prepared by the developers themselves substantiating their efforts to establish the need for an herbicide tolerant GM crop and thereby justifying the use of GM mustard as HT crop.
3. The Government, unfortunately, took a stand against this naked truth and is deceptively and unscientifically trying "not to term GM mustard as HT crop", presumably to protect the regulators, protect the interest of the developer and bypass the needed regulation. The main beneficiary of this untruthful stand of the Government would certainly be the corporate bodies in the field of herbicide business.
4. Use of double enhancer promoter in Barstar gene construct is meant for a tenfold higher expression of HT trait, which has no other justification than creation of a HT crop in the first instance.
5. CGMCP has developed and all set for developing a series of herbicide tolerant mustard lines/ cultivars, which eventually will increase the use of herbicides in Indian agriculture and thereby profiting agro-chemical MNCs as all the four herbicides (Basta, Pursuit, Algrip and Round Up) are largely marketed / patented by MNCs-Bayer, BASF, DuPont and Monsanto respectively.

In this background, the Sálim Ali Foundation pleads with each member of the GEAC not to grant approval for the commercial release of the GM mustard proposed by the CGMCP and, declare an indefinite moratorium on it.

*** Dr. V. S. Vijayan is an ornithologist, wildlife biologist, and the founding Director of the Sálim Ali Centre for Ornithology and Natural History (SACON), a Centre of Excellence of the Government of India. He was a member of Western Ghats Ecology Expert Panel (WGEEP) known as Gadgil Committee and former Chairman of Kerala State Biodiversity Board. He has been associated with Bombay Natural History Society for more than four decades in different capacities. Currently he is the Chairman of the Sálim Ali Foundation.**

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