SOUTH ASIA
BIOTECHNOLOGY CENTRE®

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Sub: SABC's Comments on the Food and Environment Safety Assessment (AFES) Report on Barnase-Barstar GE Mustard

Ref: Assessment of Environment and Food Safety (AFES) for the environmental release of genetically engineered mustard (Brassica juncea) hybrid DMH-11 and use of parental events (Varuna bn 3.6 and EH-2 modbs 2.99) for development of new generation hybrids

Dear Dr. (Mrs.) Amita Prasad

In response to MOEF's call for comments on Safety Assessment Report on GE Mustard submitted by Sub-Committee to GEAC, the South Asia Biotechnology Centre (SABC) - a registered not-for-profit scientific society is pleased to make following observations and comments with respect to the-state-of-the-art *barnase-barstar* GE mustard technology, which is being developed by the Brassica group of the University of Delhi South Campus. Following comments are the synthesis of a thorough review of the AFES of GE mustard document by a group of multi- and interdisciplinary experts comprising of scientific & researchers, breeders, biotechnologists, geneticists, plant pathologists and physiologists, seed technology and production experts, farm policy, economics and extension experts, intellectual property experts and people familiar with farm and farming system of mustard in the country.

At the outset, we would like to congratulate the brassica team at Delhi University for perfecting a system of the *barnase-barstar* GE technology that is aimed at developing an efficient hybrid seed production system in widely cultivated Indian mustard (*Brassica juncea)*. We are also pleased to note that DMH-11 hybrid based on the *barnase-barstar* system, is the first public sector mustard hybrid developed indigenously with the funding of the Department of Biotechnology (DBT) of the Ministry of Science and Technology (MOST) and the National Dairy Development Board (NDDB) – the largest producer and supplier of Dhara branded edible oil in India. Notably, the Brassica group at Delhi University South Campus was also the first group in the country to identify and develop the cytoplasmic male sterility (CMS) based conventional mustard hybrid DMH-1 way back in early 2000. Subsequently, many public and private sector institutions have developed CMS based mustard hybrids, however, constrained with various limitations associated with CMS systems.

Our comments on the development of GE mustard are captured with respect to multiple parameters including the global overview of *barnase-barstar* technology; 20 years of successful cultivation of *barnase-barstar* GE canola; significance of barnase-barstar technology in the global trade of GE edible oilseed grains, cooking oil and animal feed; advancement of GE technology in edible oilseed crops; science of *barnase-barstar* GE system; importance of *barnase-barstar* mustard for farmers' competitiveness and a robust national edible oil economy; superiority of *barnase-barstar* system over the conventional CMS based mustard hybridization system; 20 years of history of safe use of *barnase-barstar* GE canola and yield advantage of *barnase-barstar* GE mustard over conventional mustard.

Finally, we append a summary of the scientific evidences and risk-benefit analysis on different topical issues particularly related to the barnase-barstar GE mustard technology. Also enclosing comments in the proforma as prescribed by the MOEF&CC. It is important to note the our policy makers and society-at-large have been swayed and misled with emotive issues, misconception, fear and obfuscated lies by a section of anti-farm innovation groups interested in promoting imported GE canola and GE soybean cooking oil to derail this important GE mustard farm innovation that can help our mustard farmers to produce more mustard per unit area, substantially increase their income and contribute to overall increase in domestic mustard production in India.

Global Overview of *Barnase-Barstar* Technology:

Madam Chairperson, it is important to note that related species of genus Brassica, *Brassica napus* popularly known as canola expressing *barnase-barstar* genes was approved for commercial cultivation since 1996. Globally, there are as many as 26 events of GE canola (*Brassica napus*) approved for commercial cultivation in Canada, USA and Australia from 1996 to 2016. In principal, the commercially approved GE canola expresses single and/or stacked traits of herbicide tolerance and pollination control system required for commercial exploitation of heterosis and, stacking of multiple events of glyphosate tolerance, glufosinate tolerance and pollination control system etc. Many countries have also approved the consumption of GE canola grains, cake and oil for the food, feed and processing (FFP). Countries importing GE canola products include Japan, Korea, China, Taiwan, Philippines, New Zealand, European Union, South Africa, Chile and Mexico (Table 1). Notably, India is one of the major importers and consumers of GE canola oil, approx. one and half million tons per annum from Canada and USA but has not official issued import approval yet.

Table 1. Status of Commercial Approval of GE Canola (*Brassica napus*), 1996 to 2016

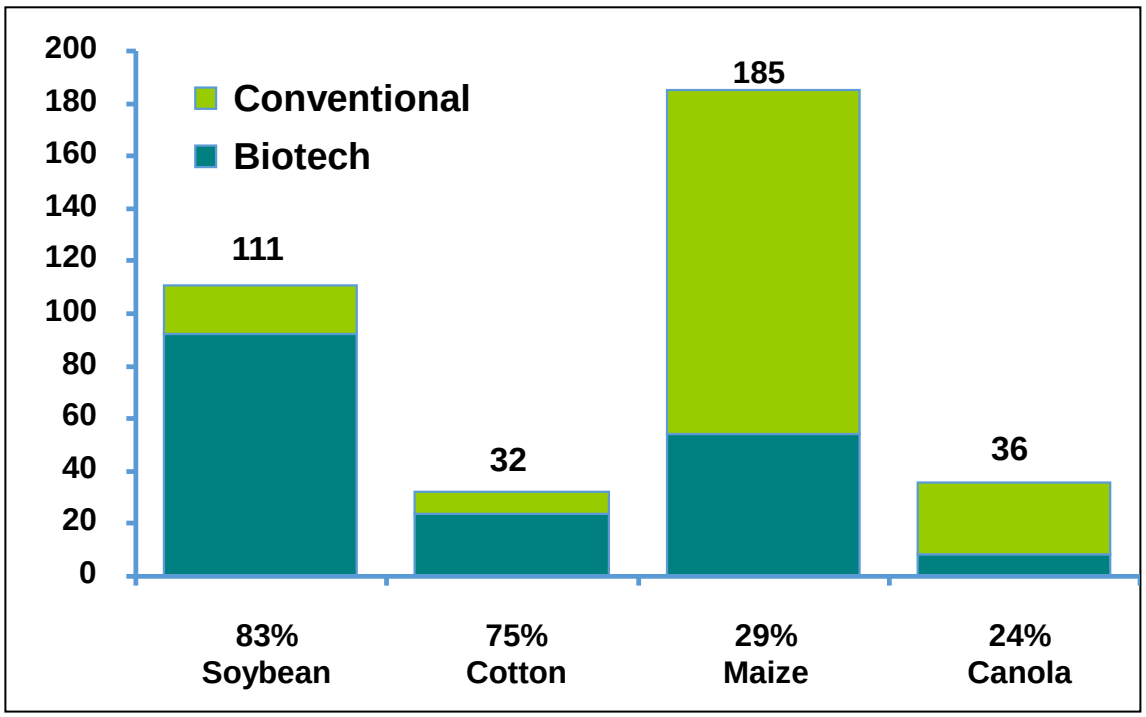
Trait/Stacked Trait(s)	Gene(s)/ Herbicide	Nos of Approved Events	Developers/Trade Name	Commercial Approval	Approval for food and Feed Consumption
-Multiple Mode Herbicide Tolerance -Pollination Control System -Stacked Herbicide Tolerance & Pollination Control System	Bar, Barnase & Barstar; Glyphosate; Glufosinate	26	-Bayer CropScience -Monsanto -Pioneer/Dupont (InVigor; LibertyLink; Navigator; TruFlex; Roundup Ready)	Canada; USA; Australia	Australia; Canada; Chile; China; European Union; Japan; Mexico; New Zealand; Philippines; South Africa; South Korea; Taiwan & USA

Source: Analysed by SABC, 2016

20 Years of Successful Cultivation of Barnase-Barstar GE Canola:

Around a quarter of global Brassica area equivalent of 8.5 million hectares or 24% of total Brassica area of 36 million hectares is genetically modified in 2015. It needs mention that Canada and USA have been benefiting from GE canola since 1996. Australia has been growing GE canola since 2008. In 2015, Canada grew GE canola over 7.4 million hectares or 92.5 % of total canola area whereas, USA grew GE canola over 591,000 hectares or 93% of 636,000 hectares of canola area in the country. Similarly, Australia has gradually increased area under GE canola to 444,000 hectares or 22% of 2 million of canola grown in three States of New South Wales (NSW), Victoria and Western Australia (Figure 1). In summary, **GE canola is commercially cultivated over an area equivalent to one and half times the area under mustard in India**. It is estimated that GE canola has contributed immensely in increasing the income benefits for farmers by US$ 4.9 billion between 1996 and 2014.

Figure 1. Global Adoption of GE Soybean and GE Canola (Mha), 2015

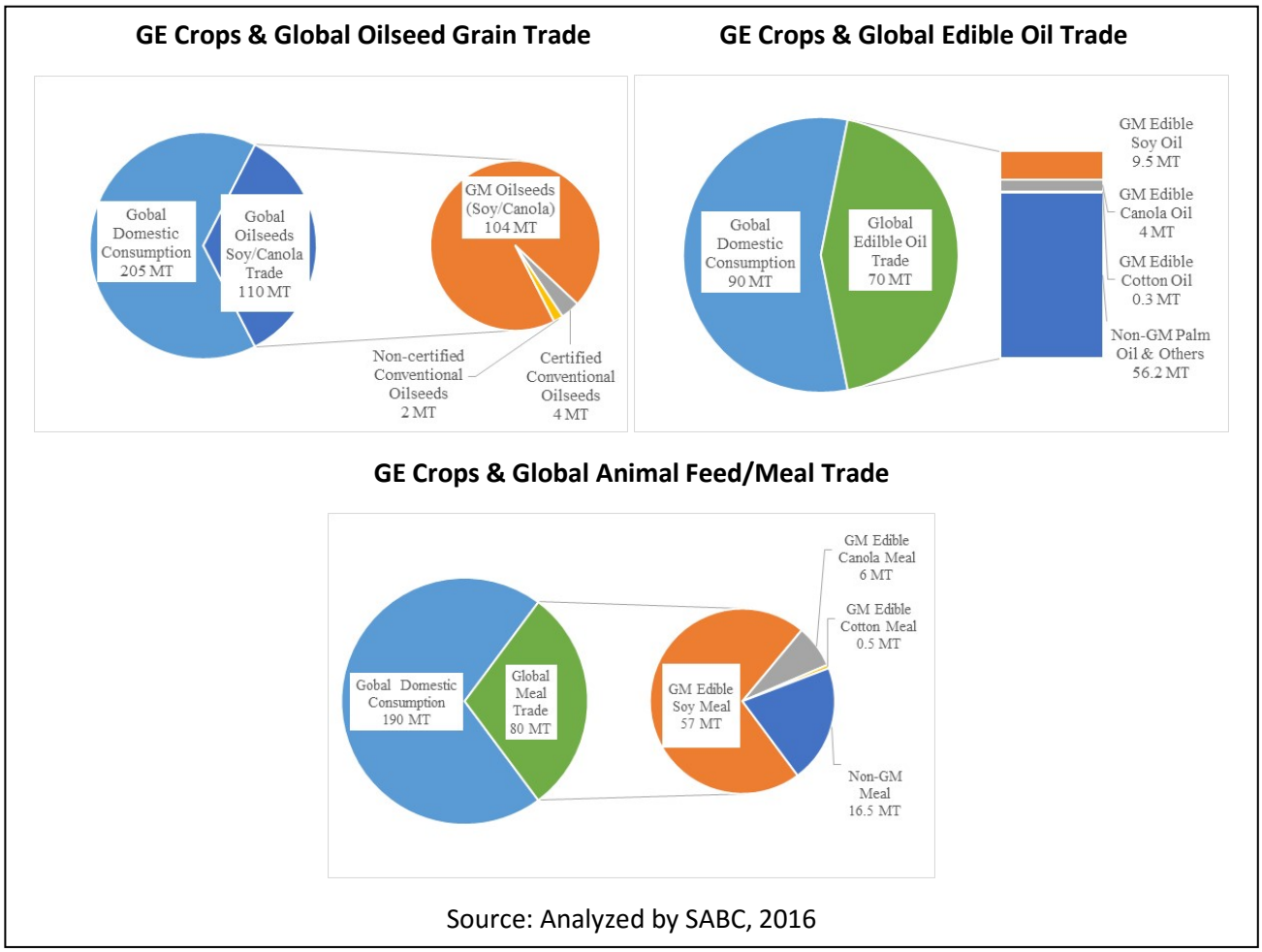


Source: Clive James, 2016 (Hectarage based on FAO data on 2013)

Significance of *Barnase-Barstar* Technology in the Global Trade of GE Edible Oilseed Grains, Cooking Oil and Animal Feed:

Canada, USA and Australia dominate the global trade in Brassica grain, cake and edible oil, which is either derived from or containing GE canola. In 2014, GE canola contributed 12 million tons to the global grain trade, 4 million tons to global edible oil trade and 6 million tons to global animal feed/cake trade. Noticeably, GE soybean and GE canola account approx. 104 million tons or 94 % of 110 million tons of global oilseeds trade in soybean and canola; 15 million tons or 22 % of 70 million tons of global edible oil trade and 64 million tons or 80 % of 80 million tons of global animal feed/cake trade. Therefore, it is evident that GE soybean and GE canola contribute a major portion of the global trade in edible oilseeds, edible oil and animal meal/cake (Figure 2).

Figure 2. Impact of GE Soybean and GE Canola on the Global Trade in Oilseeds Grains, Edible Oil and Animal Feed, 2014



Source: Analysed by SABC, 2016

Advancement of GE Technology in Edible Oilseed Crops:

In addition to GE canola, SU Canola™ - a new gene-edited crop developed by Cibus was commercialized for the first time and planted on an estimated 4,000 hectares in the US in 2015. The SU Canola™ imparts resistance to a new herbicide sulfunyl urea. The SU Canola™ was developed using precision gene-editing technology such as the Oligonucleotide directed mutagenesis (ODM) and the Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR). The SU Canola™ was not subjected to regulatory approval under the US Coordinated Biotechnology Regulatory Framework. There are 20 canola events of SU Canola approved for food, feed and cultivation in the USA as of October 2015. We expect that the gene-edited technologies will be increasingly applied in conjunction with GE traits to improve the quality of fatty acid composition of different edible oil including GE Soybean and GE canola in the future.

Science of Barnase-Barstar GE System:

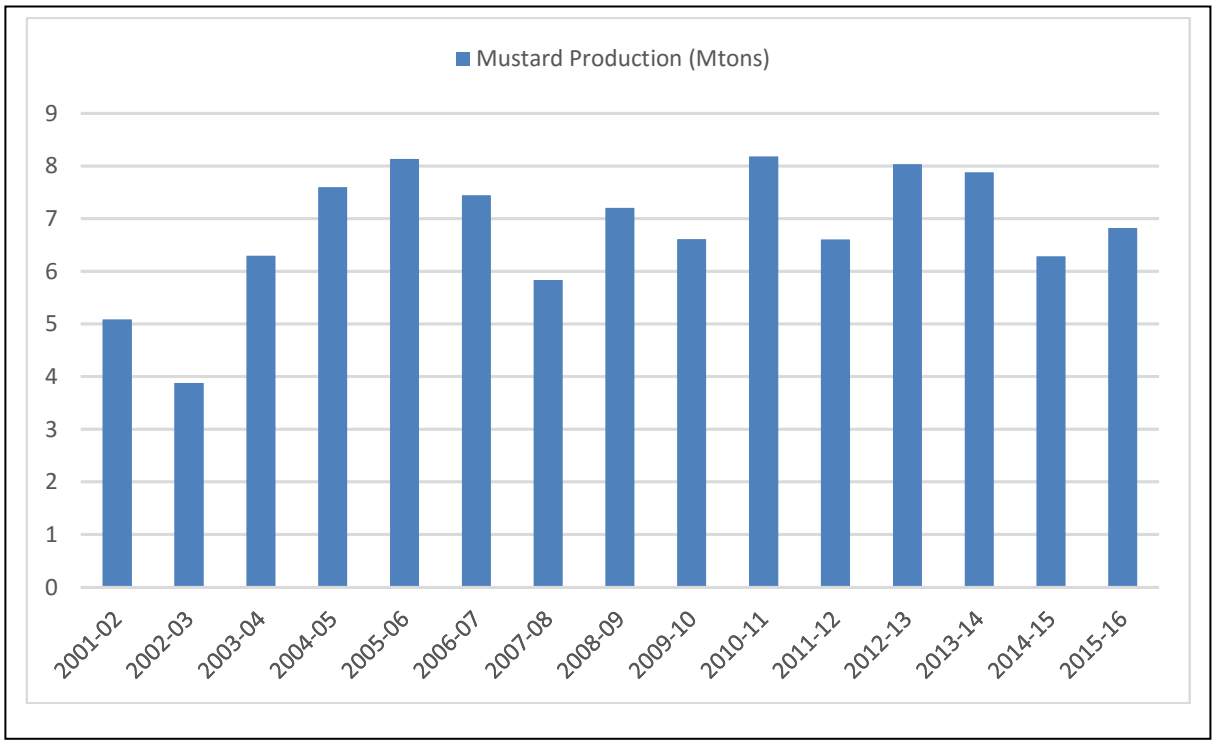
Madam Chairperson, the AFES of GE document eloquently described the *barnase-barstar* pollination control system in Indian mustard. DMH-11 mustard hybrid is a combination of the event bn 3.6 (Varuna Barnase line) and event modbs 2.99 (EH-2 Barstar line) which were selected in RLM 198 and Varuna variety of *B. juncea* respectively by University of Delhi South Campus. The event bn 3.6 and modbs 2.99 were backcrossed into variety Varuna and east-European type mustard line EH-2 respectively, which were further crossed to form hybrid DMH-11 expressing events bn 3.6xmodbs 2.99. We are satisfied with a thorough and rigorous regulatory assessment of GE mustard hybrid DMH-11 with respect to biosafety, efficacy, hybridization, crossability and field performance under the Indian regulatory framework over the period of more than 10 years. Importantly, GE mustard DMH-11 hybrid was assessed under multiple field trials for the field level performance in close collaboration with ICAR-DRMR and other Brassica research groups at IARI, New Delhi and PAU, Ludhiana. Notably, the biosafety studies including the crossability study of the *barnase-barstar* mustard hybrid DMH-11 with related Brassica species such as *B.rapa* (toria, yellow sarson, brown sarson), *B.nigra*, *B.oleracea* (early types), *B.napus*, *B.carinata*, *B.touneforti*. *Eruca sativa* and *Raphanus sativus* reinforces the environmental safety with respect to gene flow and its consequences on the environment. We have also noted that the requisite multiple field trials were conducted in the major mustard growing States of Rajasthan, Haryana, Punjab and Delhi, which cultivate more than 90% of mustard in India.

Importance of *Barnase-Barstar* Mustard for Farmers' Competitiveness and a Robust Edible Oil Economy:

Madam Chairperson, India faces a huge deficit in edible oil production and annually imports approx. 14.5 million tons of edible oil including oil extracted from GE soybean and GE canola. Annually, **India spends approx. US$12 billion equivalent to Indian Rupee 78,000 crores on imported edible oil growing at double digits to meet the burgeoning domestic requirement**. The edible oil deficit will continue to widen with the increase in the population and per capita income. To address this insurmountable challenge, India needs to critically look into ways and means to increase productivity of oilseed crops including mustard, soybean and cotton. GE mustard hybrid DMH-11 is one of the promising technologies to improve mustard yield in India, which is almost stagnant since last two decades. Figure 3 & 4 shows the trend in mustard yield & production in India, 2000 to 2015.

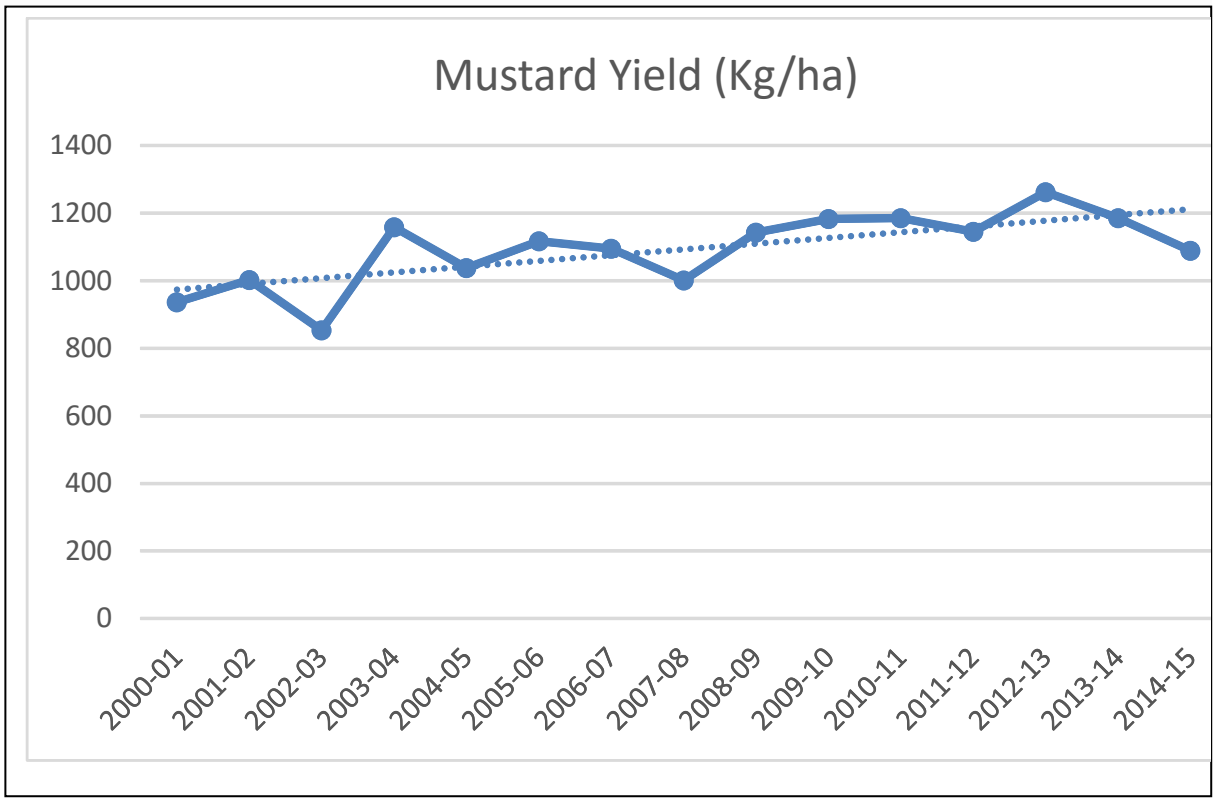
Madam Chairperson, mustard is one of major edible oilseed crops followed by soybean, groundnut, sunflower, castor, sesamum, safflower, linseed and niger seed. Of the 28 million tons of oilseeds produced in 2015-16, soybean and mustard contributed 8.6 million tons and 6.8 million tons respectively. Mustard yield hovers around 1000 kg per hectare (half of world's average) irrespective of improved agronomy, high seed replacement rate and irrigation facilities. **Ironically, mustard production and yield remains stagnated for the last two decades amidst the release of 96 varieties of Indian mustard since the inception of ICAR AICRP-RM from 1967 to 2014**. In addition, both public and private sector have extensively used CMS systems of *Ogura* and *Moricandia arvensis* cytoplasm in the production of conventional mustard hybrids. However, a narrow variability in mustard germplasm of *Brassica juncea* is inadequate in breaking the existing yield ceiling. Additionally, mustard production is severely constraints by multiple factors including biotic and abiotic stresses including white rust, Alternaria blight tolerance, Sclerotinia rot and Orobanche etc.

Figure 3. Trend in Mustard Production in India, 2001 to 2015



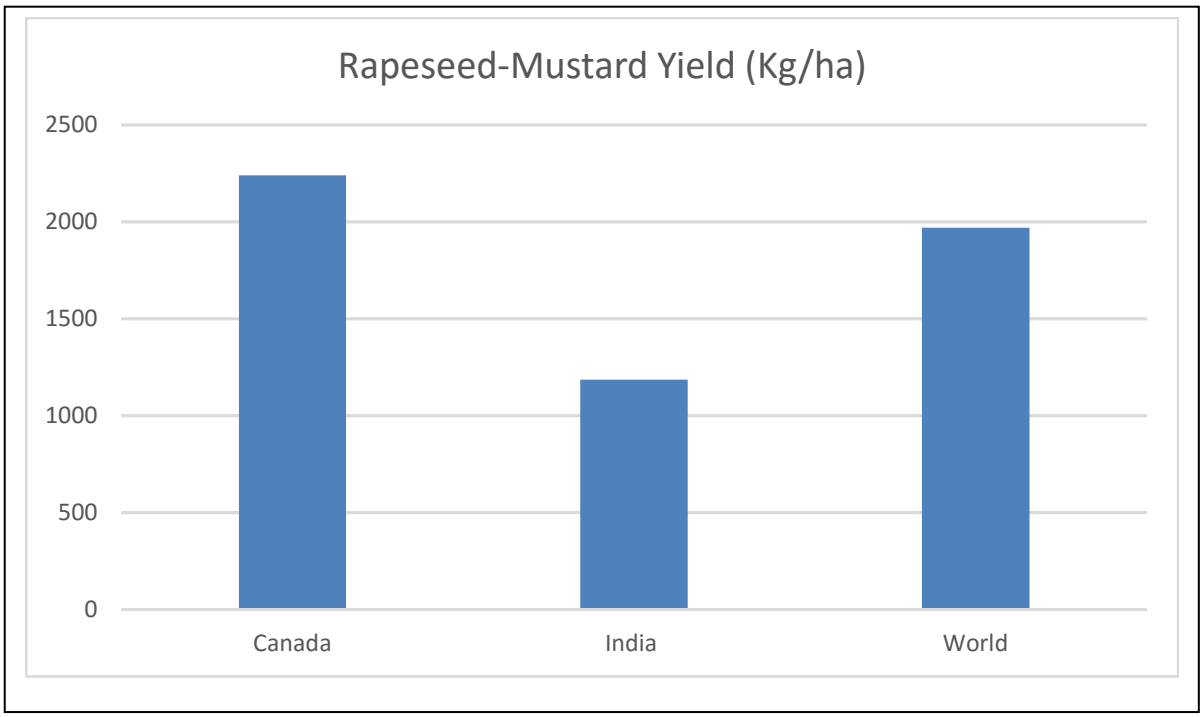
Source: MOA&FW, 2015; Analyzed by SABC, 2016

Figure 4. Trend in Mustard Yield in India, 2001 to 2015



Source: MOA&FW, 2015; Analyzed by SABC, 2016

Figure 5. Yield Comparison of Rapeseeds-Mustard, 2014-15



Source: USDA, 2015; Analyzed by SABC, 2016

Superiority of *Barnase-Barstar* System over the Conventional CMS based Mustard Hybridization System:

In the past, the conventional mustard hybrid production system using cytoplasmic male sterility (CMS) system, a non-GE method for pollination control has been used to produce mustard hybrids in India. **These CMS-Fertility restoration system suffers from low purity & incomplete fertility restoration in the hybrids and pollen shedders in the sterile line due to break down of the system imposed by genetic and environmental reasons**. The *barnase-barstar* system of mustard hybridization is very versatile, which will accelerate the mustard breeding program in India.

20 Years of History of Safe Use of *Barnase-Barstar* GE Canola:

Madam Chairperson, different studies conducted in India, along with earlier research studies from USA, Canada, Australia, Japan and EU have shown that proteins encoded by the *barstar-barnase* genes are neither allergenic nor toxic. Edible oil and meal extracted from GE canola is a major source of edible oil for food and animal feed in the world. Notably, GE hybrid DMH-11 seed does not contain any barnase or barstar protein, except that the bar protein is present at very low levels with almost no significance at field level. Edible oil extracted from GE mustard does not contain any of the three proteins, and therefore it is completely safe for human consumption.

Yield Advantage of Barnase-Barstar GE Mustard Over Conventional Mustard:

Madam Chairperson, the multiple field trials of DMH-11 over different locations, as tabulated in AFES document demonstrate that GE mustard hybrid DMH-11 yields significantly higher than the popular mustard varieties in India. On average, GE DMH-11 hybrid has shown yield superiority of 28 per cent over the mega variety Varuna and 38 per cent over the control varieties. Given the high seed replacement rates (SRR) in mustard, around 71% at the national level and almost 100 % in some of the States like Gujarat, the deployment of the *barnase-barstar* system will open a new opportunity in improving mustard productivity and production necessary to narrow down the edible oil deficit.

Finally, we summarize and support the scientific evidences and risk-benefit analysis on different topical issues particularly related to the barnase-barstar GE mustard technology. Also enclosing comments in proforma as prescribed by the MOEF&CC. Following sections highlight major topical issues related to the *basrnase-barstar* GE mustard technology, and our expert submissions underscore the safety, efficacy, agronomic performance and environmental sustainability of *barnase-barstar* GE mustard technology.

INCREASING MUSTARD YIELD:

The yield of conventional mustard varieties are very low - as low as one third of its counterpart GE canola hybrids grown over large scale in developed countries. Existing crop management methods and extension services have been exploited to the fullest resulting in modest yield increases in the recent past (Figure 5). In addition, public sector institutions of ICAR and SAUs have also developed suitable technologies through genetic interventions coupled with appropriate crop production and protection methodologies to increase mustard yield. Notably, majority of Indian mustard farmers have a very high seed replacement rate (SRR) of mustard seeds due to relatively better genotypes and hybrids to ensure yield optimization. High SRR is primarily due to low seed rate requirement for planting and affordable seeds. Despite all efforts, mustard yield remains low, and thus affects mustard production, profitability and prosperity of mustard farmers. Therefore, **it is paramount to introduce the *barnase-barstar* technology option to improve productivity of mustard beyond the present level** (Figure 4). Hybrids which ensure better yield performance in major crops is the most viable option to improve yield and crop profitability. In addition, new approaches such as the *barnase-barstar* system provide perfect system for low cost hybrid seed production.

EFFICIENT HYBRIDIZATION OF MUSTARD:

In the recent years, both public and private institutions have introduced mustard hybrids based on the cytoplasmic male sterility (CMS) systems. *Ogura* and *Moricandia arvensis* based CMS systems have led to development of mustard hybrids for exploiting heterosis in the mustard germplasm. However, increasing the level of heterosis in mustard hybrids is a major challenge to make the hybrid technology competitive and remunerative. In addition, there is a need to improve fertility influenced by cytoplasm and restorer gene in hybrid crop. **The barnase-barstar system provides opportunity to produce fully fertile hybrid with enhanced yield levels, reduce hybrid seed production cost and increase farmers' income**. Importantly, new GE system also provides an ease in producing hybrid seeds by reducing the complication of three lines system. With the availability of this

technology, small farmers can also efficiently produce large quantity of hybrid seeds necessary for increasing adoption of mustard hybrid seeds in the country. In addition, timely deployment of GE technologies including herbicide tolerance over time and space shall raise the genetic potential and impart resistance to major biotic and abiotic stresses to overcome yield barrier.

MUSTARD CROSSABILITY:

The biosafety studies including the crossability study of the *barnase-barstar* mustard hybrid DMH-11 with related Brassica species such as *B.rapa* (toria, yellow sarson, brown sarson), *B.nigra*, *B.oleracea* (early types), *B.napus*, *B.carinata*, *B.touneforti*. *Eruca sativa* and *Raphanus sativus* were carried out to assess the gene flow and its consequences on the environment. **The issue of crossability of GE mustard with the conventional mustard or wild relatives has been overstated and exaggerated to stall the commercial cultivation of this powerful hybridization technology**. Moreover, farmers usually grow F1 hybrid seeds season-after-season and avoid the planting of F2 seeds. Notably, over the years, the public sector institutions such as ICAR-DRMR, IARI, PAU, HAU and NBPGR have already been maintaining the genetic pool of mustard crop collected through exploration, characterized and evaluated. Importantly, the conventional commercial mustard has already been grown using improved seeds bred by public and private sector institutions. Therefore, the present situation on commercial cultivation of mustard is similar to the one which is proposed using the *barnase-barstar* technology. Once a mustard hybrid possessing the *barnase-barstar* system is accepted by Indian mustard farmers, more diverse mustard hybrids will be made available to farmers to improve mustard productivity and their farm income.

HERBICIDE TOLERANCE IN MUSTARD:

The herbicide tolerance is not a prime target for the *barnase-barstar* GE mustard hybrid DMH-11. The *barnase-barstar* system addresses the major challenge faced by breeding community to overcome issues related to efficient mustard hybrid seed production. Globally, the stacked traits of *barnase-barstar* technology with herbicide tolerant GE canola was approved for commercial cultivation since 1996. Stacked traits GE canola is most preferred by farmers to exploit heterosis and efficiently control weeds in Canada, USA and Australia. As of 2016, a quarter of global Brassica area equivalent of 8.5 million hectares or 24% of total Brassica area of 36 million hectares is tolerant to multiple herbicide including glyphosate (*Roundup Ready*™) and glufosinate (*LiertyLink*™) etc. Notably, the weeds remain one of the major constraints to mustard yield in India. ICAR-DRMR estimates that stresses caused by insect, nematodes, fungal, bacterial and viral pathogens, Orobanche and weeds collectively result in approximately 45% yield loss annually. **Therefore, all efforts should be directed to develop mustard tolerant to popular herbicides including glyphosate and glufosinate to allow farmers to increase mustard productivity and production in India**.

NON-TERMINATOR TECHNOLOGY & MALE STERLITY TRAIT IN MUSTARD:

It is noted that the *barnase-barstar* GE mustard hybrid DMH-11 does not carry gene(s) imparting Genetic Use Restriction based Technology (GURT), popularly known as terminator technology. As we know, Indian regulatory system neither allow use of GURT for research nor for developing new plant material for commercial purpose. **Efforts should be**

made to ensure that the general public should not be confused with the system of male sterility induced by the *barnase-barstar* technology with the GURT and terminator technology.

The system of male sterility in one of the parents is a fundamental necessity for efficient hybrid seed production irrespective of use of methodologies either the cytoplasmic male sterility (CMS) or the *barnase-barstar* system. Moreover, the cytoplasmic male sterility has been widely used for producing hybrid seeds in different crops including mustard. Majority of conventional mustard hybrids are based on the cytoplasmic male sterility (CMS) systems of *Ogura* and *Moricandia arvensis* cytoplasm.

Underscoring the potential of the *barnase-barstar* GE mustard technology, the South Asia Biotechnology Centre (SABC) strongly recommends that GEAC/MOEF&CC should be considerate in evaluating the benefits of this important breeding tool for the benefits of the scientific and farming community. **May we appeal to GEAC/MOEF&CC to immediately declare the *barnase-barstar* technology as safe for environmental release and approve hybrid DMH-11 for commercial cultivation**. The *barnase-barstar* system shall allow our Brassica breeding teams at ICAR-DRMR, IARI, HAU and PAU and other groups to easily produce high yielding mustard hybrids and expeditious delivery of this new farm innovations from the lab to the land.

The dream of our honourable Prime Minister Narendra Modi Ji is to double farmers' income by 2022 can be realized provided that GEAC/MOEF&CC is considerate in timely release of *barnase-barstar* GE mustard and other important farm innovations pending commercialization in order to increase farm productivity, farmers' profitability and agriculture sustainability.

We shall be pleased to engage and share our views and insights with GEAC/MOEF&CC for the growth of mustard and agriculture sector in India.

Thanking you for the opportunity.

Sincerely yours,

C.D. Mayee
President & Founder Director

Bhagirath Choudhary
Founder Director

For and on the behalf of the Board of Directors of the South Asia Biotechnology Centre