

Safety Assessment of GM Mustard Hybrid

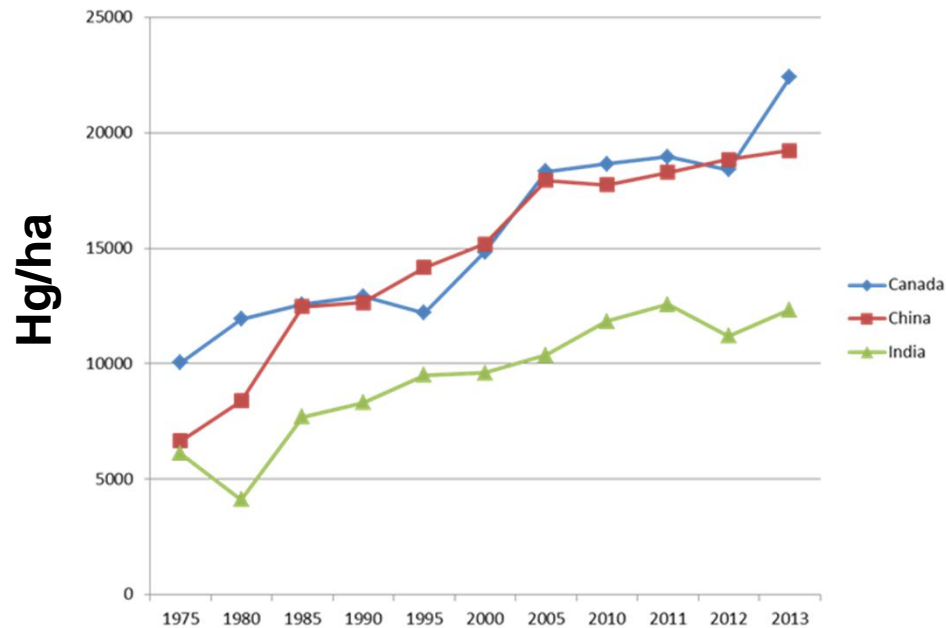
A Case Study



Economic importance of mustard

- ❑ Mustard is one of the major oilseed crop of India grown in around 6-7 MHa of land
- ❑ India has emerged as the largest importer of vegetable oil
- ❑ Domestic production is half of total consumption

Three country comparison of rapeseed mustard yield



Development of GM mustard hybrid DMH-11

- ❑ GM mustard hybrid, DMH-11, has been developed by CGMCP, University of Delhi South Campus
- ❑ Trait introduced is male sterility and fertility restorer using *barnase/barstar* genes and *bar* gene has been used as a selectable marker
- ❑ The technology was ready by 2002 with funding from National Dairy Development Board and DBT
- ❑ Patented in US, Canada and India
- ❑ Field evaluation undertaken for 2 years after seeking regulatory approvals, primarily to study the efficacy of the system
- ❑ Trials conducted for two years by ICAR to check performance of hybrid
- ❑ The hybrid showed 20-30% heterosis over check varieties

Functions of the introduced genes

Genes	Codes for	Source	Trait
<i>barnase</i>	Barnase ribonuclease	<i>Bacillus amyloliquefaciens</i>	Male Sterility
<i>barstar</i>	Barnase ribonuclease inhibitor	<i>Bacillus amyloliquefaciens</i>	Fertility restorer
<i>Bar</i> *	Phosphinothricin acetyltransferase (PAT)	<i>Streptomyces hygroscopicus</i>	Marker

***The expression of the *bar* gene is not intended for herbicide tolerance; will be used only for selection of lines during the seed production.**

**Normal male fertile flower
(Non transgenic)**



November 3, 2015

**Male sterile flower with
barnase gene
(Transgenic)**

Regulatory approval status of GM canola with *barnase/barstar* system

Event	Country	Environment	Food and Feed	Food	Feed
ACS-BNØØ4-7 x ACS-BNØØ1-4 (MS1, RF1 =>PGS1)	Australia	2003	2002		
	Canada	1995		1995	1995
	China		2004		
	European Union		2005		
	Japan	1996		1996	1996
	Korea			2005	2008
	South Africa		2001		
	United States	2002	1996		
ACS-BNØØ4-7 x ACS-BNØØ2-5 (MS1, RF2 =>PGS2)	Australia	2003	2002		
	Canada	1995		1995	1995
	China		2004		
	European Union		2005		
	Japan	1997		1997	1997
	Korea			2005	2008
	South Africa		2001		
	United States	2002	1996		
ACS-BNØØ5-8 x ACS-BNØØ3-6 (MS8xRF3)	Australia	2003	2002		
	Canada	1996		1997	1996
	China		2004		
	European Union		2005		
	Japan	1998		1997	1998
	Korea			2005	2005
	Mexico		2004		
	South Africa		2001		
United States	1999	1996			

Studies conducted on DMH-11 and its parental lines

Event generation and Molecular characterization	<ul style="list-style-type: none">➤ Gene sequences, constructs, transformation and molecular characterization➤ Expression studies for the inserted genes
Food/feed safety studies	<ul style="list-style-type: none">➤ Cloning, expression, purification and production of expressed proteins in heterologous system➤ Acute oral toxicity with pure protein➤ Sub-chronic toxicity with whole grain/edible plant parts➤ Compositional analysis
Allergenicity Studies	<ul style="list-style-type: none">➤ Bioinformatics analysis➤ Pepsin digestibility➤ Heat stability
Environmental safety studies	<ul style="list-style-type: none">➤ BRL-1 field trials for two years and BRL-II trials for one year➤ Weediness potential and aggressiveness parameters➤ Crossability and pollen flow studies➤ Pollination behavior, pollen morphology and physiology➤ Impact on soil microflora
Detection protocols	<ul style="list-style-type: none">➤ Protocol for testing at a level of detection (LOD) of 0.01%➤ Development of ELISA kits

BRL-I & II confined field trials

- Conducted under the Coordination of Directorate of Rapeseed-Mustard Research, Bharatpur During Rabi 2010 and 2011, 2014

- Lines tested
 - i. Varuna Barnase bn 3.6 *bar, barnase*
 - ii. EH2 Barstar modbs 2.99 *bar, barstar*
 - iii. Varuna (also a national check)
 - iv. EH2
 - v. DMH-11 *bar, barnase, barstar*
 - vi. RL 1359/ Maya (local checks)

Mean Seed yield (Kg/Ha) of DMH-11 (BRL- 1 and 2)

S No	Entry	Mean Seed Yield kg/ha			Overall Mean	% Increase over
		2010-11	2011-12	2014-15		
1	Varuna	2093	2617	1887	2199	28.41
2	Varuna (barnase)	2096	2640	1861	2199	
3	EH-2	1897	2007	1378	1761	
4	EH-2 (barstar)	2009	1856	1558	1808	
5	Maya/RL-1359 (ZC)	2037	2323	1776	2045	38.05
6	DMH-11	2600	3485	2386	2824	

Studies undertaken by CROs

Activities	CRO
Cloning and purification and Production of sufficient quantities of pure proteins Bar, Barnase and Barstar	Premas Biotech, Gurgaon
Development of ELISA kits for the three proteins	Amar Immunodiagnostics Hyderabad
Assessment of possible Allergenicity (bioinformatics studies), heat stability and pepsin digestibility	NIN, Hyderabad
Acute oral toxicity studies and sub-chronic toxicity studies	NIN, Hyderabad
Compositional analysis	NIN, Hyderabad
Impact on soil microflora	IMTECH, Chandigarh