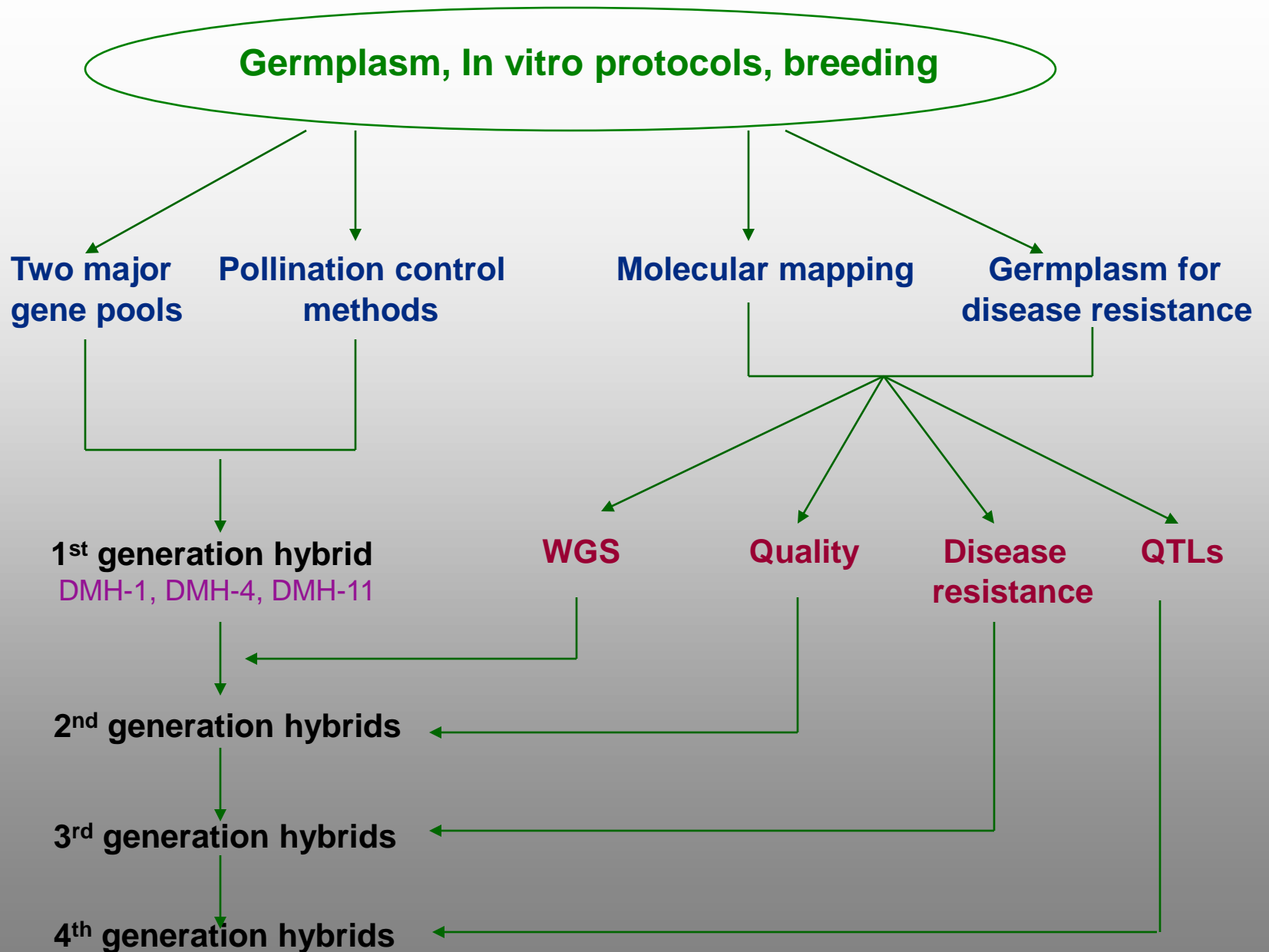


Objective Based Mustard Breeding



Breeding of Oilseed Mustard



Productivity enhancement

- Yield per se

Hybrids

- Yield protection

Aphids

GM

White rust

CB

Alternaria blight

GM

Stem rot

CB/GM

Orobanche

GM

- Quality

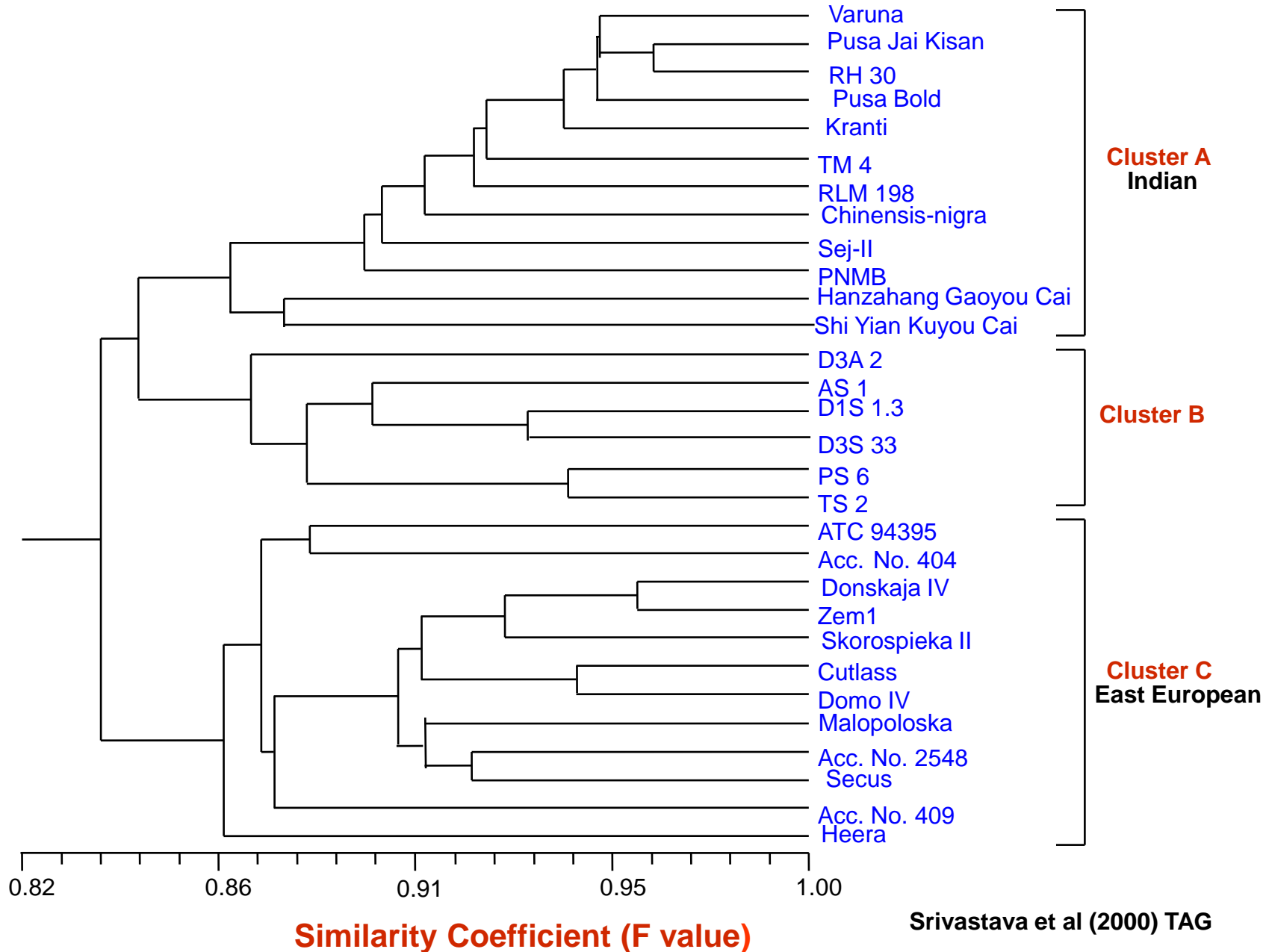
CB & GM

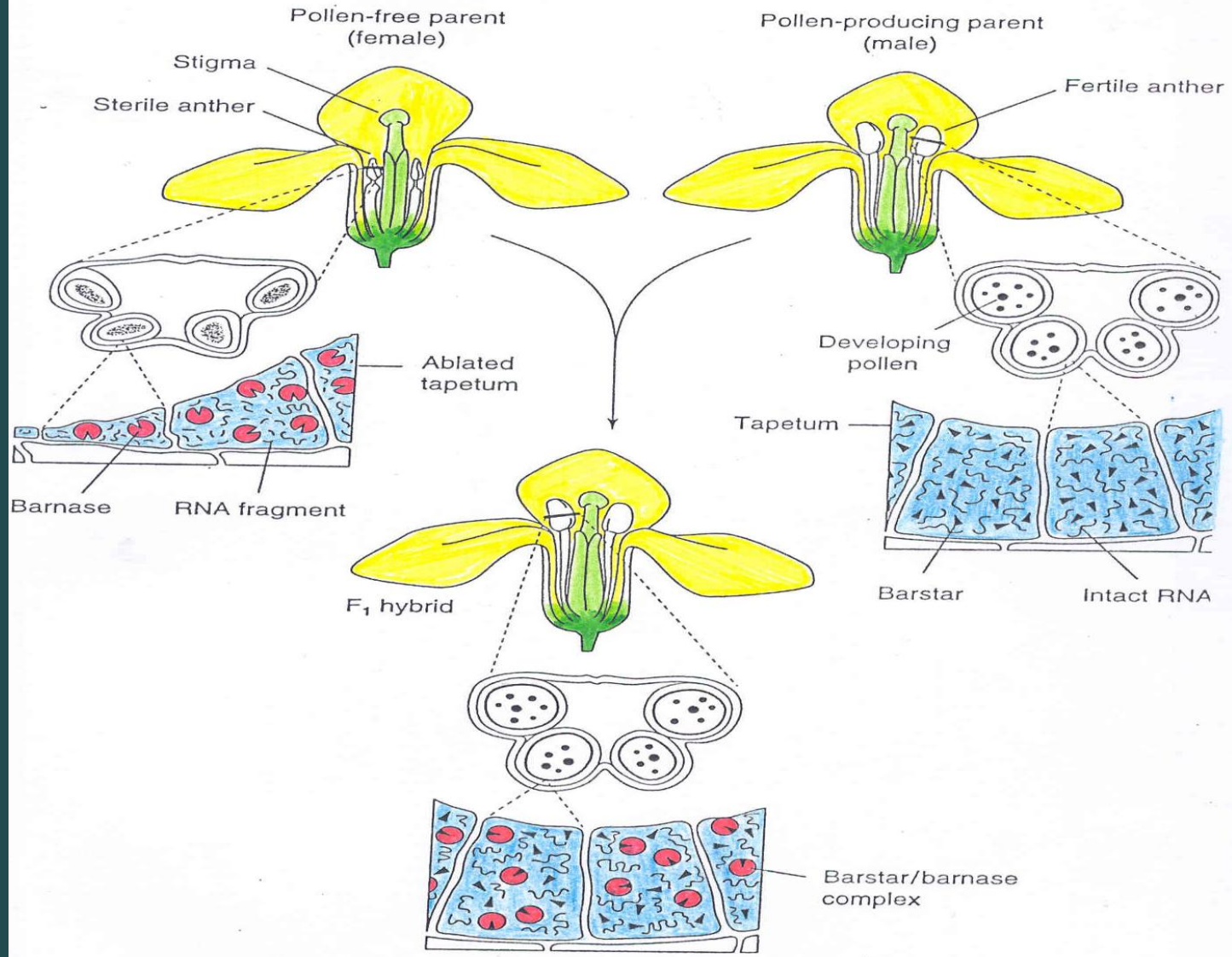
CB: conventional breeding, GM: transgenics

Components of Heterosis Breeding

- **Good combiners**
- **Pollination control mechanism**

Two Major Gene Pools in *B. juncea*





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



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

List of studies undertaken for safety assessment of Varuna bn 3.6, EH-2 modbs 2.99 and DMH-11

Molecular characterization	<ul style="list-style-type: none"> ➤ Gene sequences, constructs and molecular characterization ➤ Expression studies of the three inserted genes – <i>bar</i>, <i>barnase</i> and <i>barstar</i>
Food safety studies	<ul style="list-style-type: none"> ➤ Cloning, expression, purification and production of three expressed proteins ➤ Equivalence of the Bar, Barnase and Barstar recombinant proteins produced in bacteria with that expressed in transgenic plants ➤ Bioinformatics analysis of the three proteins ➤ Pepsin digestibility of the three proteins ➤ Heat stability of the three proteins ➤ Acute oral toxicity of the three proteins in mice ➤ Sub-chronic toxicity of leaves and seeds containing the three proteins in rats ➤ Compositional analysis
Environmental safety studies	<ul style="list-style-type: none"> ➤ Field trials from 2004 – 2007 ➤ BRL-I trials for two growing seasons (2010-11, 2011-12) ➤ BRL-II trials for one growing season (2014-15) ➤ Weediness potential and aggressiveness parameters ➤ Impact on soil microflora during BRL-I and BRL-II trials ➤ Crossability and pollen flow studies ➤ Pollination behavior, pollen morphology and physiology
Detection protocols	<ul style="list-style-type: none"> ➤ Protocol for testing at a level of detection (LOD) of 0.01% ➤ Development of ELISA kits for Bar, Barnase and Barstar

Chronology of hybrid development

S. No.	Year	Activities
1	2002	Technology ready
2	2003-04 2004-05	Limited field trials at DU farm, hybrid seed production and seed multiplication
3	2005-06 2006-07	Two years multisite trials conducted at 10 locations under the supervision of NRCRM (now DRMR), Bharatpur
4	2010-11 2011-12	Two years of BRL-1 trials at three locations in Rajasthan conducted under the supervision of DRMR, Bharatpur
5	2014-15	BRL-II trials at three locations - 2 in Punjab and one in Delhi conducted under the supervision of DRMR, Bharatpur
6	2015	Submission of biosafety dossier to GEAC for environmental release



BRL-I Trial, Kumher



Crossability study at Bawana



BRL-I Trial, Sri Ganganagar



BRL-I Trial, Navgaon

Monitoring Team Visits BRL-II Trials (2014-15)



Mean seed yield (Kg/Ha) of DMH-11 (BRL-1 & BRL-II)

S No	Variety	Mean Seed Yield kg/ha			Overall Mean	% Increase over
		2010-11	2011-12	2014-15		
1	Varuna	2093	2272	1887	2084	28
2	Varuna (barnase)	2096	2291	1861	2083	
3	EH-2	1897	1741	1378	1672	
4	EH-2 (barstar)	2009	1611	1558	1726	
5	Maya/RL-1359 (ZC)	2037	2016	1776	1943	37
6	DMH-11	2600	3025	2386	2670	

Summary of the environmental safety study results

- **No variations observed on the seed germination and seedling vigour between the transgenic and their non-transgenic comparators**
- **No interspecific crossability observed with ten different species**
- **50 meters of isolation distance sufficient as no pollen flow observed beyond 20 meters distance**
- **Scanning Electron microscopy showed no differences in the exine patterns of pollen grains and seed set**
- **Soil impact studies show that soil microbial flora is not altered negatively by the cultivation of transgenic mustard**
- **No leaching of any of the three proteins observed in soil**

Summary of food/feed safety studies

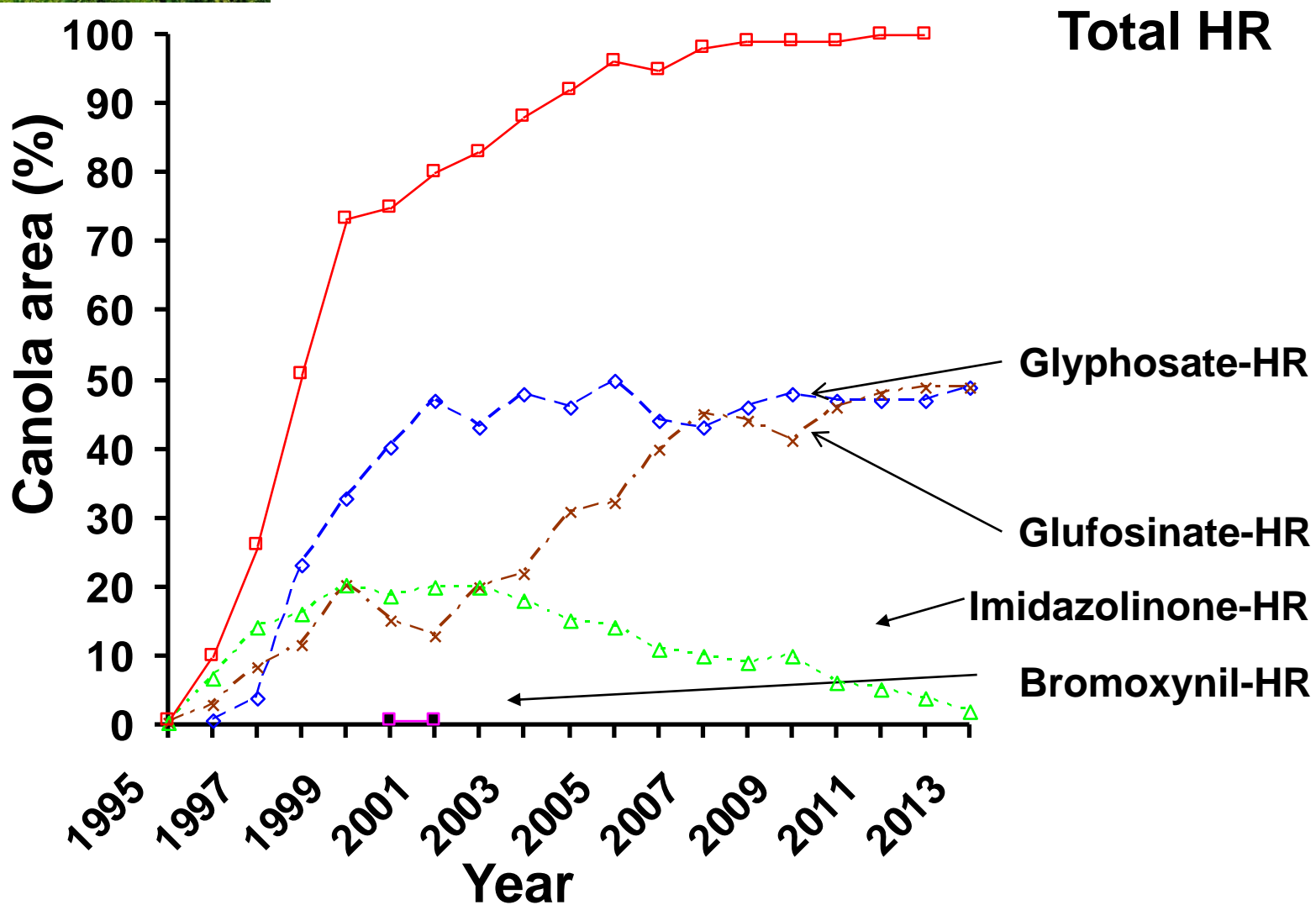
- **No sequence similarity of any of the three proteins to any allergenic proteins**
- **All the three proteins are rapidly degraded in mammalian digestive system**
- **Single dose acute toxicity studies with all the three proteins do not show any adverse effect and no mortality has been observed in mice fed with these proteins**
- **90 day sub-chronic toxicity studies using edible plant parts i.e. leaves and seeds do not show any adverse effects**
- **Compositional analysis established substantial equivalence of the transgenic vs non-transgenic mustard**

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		
	United States	1999	1996		



HR canola: 7.7 M ha (19 M ac) in 2013



Seed exports (Historic) - Canada

Historic Canadian Canola Seed Exports - updated March 28, 2012 (000 Tonnes)										
Calendar Year (January - December), Crop Year (August 1 to July 31)										
	2002 Cal. Yr.	2003 Cal. Yr.	2004 Cal. Yr.	2005 Cal. Yr.	2006 Cal. Yr.	2007 Cal. Yr.	2008 Cal. Yr.	2009 Cal. Yr.	2010 Cal. Yr.	2011 Cal. Yr.
Bangladesh	0	13	22	23	87	106	87	105	187	152
China	66	319	269	310	391	601	1476	3140	1520	1334
EU-27	8	2	1	0	1	2	2	96	129	337
Japan	1508	1681	1620	1885	1923	1915	2197	1961	2107	2328
Mexico	489	693	1081	1009	1045	1114	1282	1073	1485	1443
Pakistan	0	398	4	97	443	420	94	387	703	813
U.A.E.	0	0	0	34	330	217	505	306	773	803
U.S.A.	135	112	442	440	727	630	961	560	551	668
Others	1	22	1	2	176	10	56	2	13	12
TOTAL	2206.0	3241.5	3440.1	3797.4	5122.1	5014.1	6661.0	7630.2	7469.7	7890.0

*Numbers may be off due to rounding.

Source: Canadian International Merchandise Trade Database - Statistics Canada

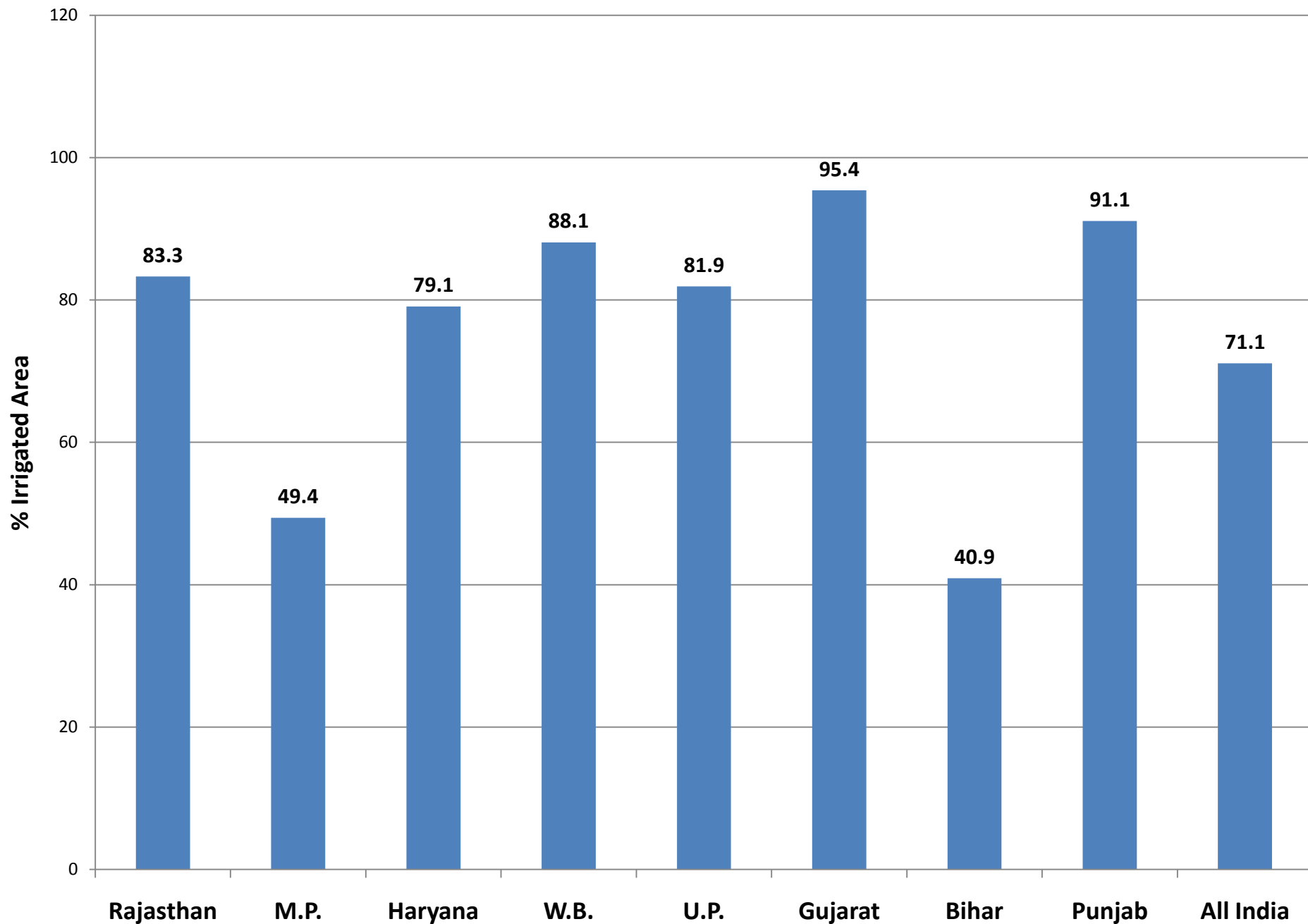
Meal exports (Historic) - Canada

Total Canadian Canola Meal (230641 + 230649) Exports - updated February 7, 2014 (000 Tonnes)												
Calendar Year, Crop Year - August 1st to July 31st												
	2002 Cal. Yr.	2003 Cal. Yr.	2004 Cal. Yr.	2005 Cal. Yr.	2006 Cal. Yr.	2007 Cal. Yr.	2008 Cal. Yr.	2009 Cal. Yr.	2010 Cal. Yr.	2011 Cal. Yr.	2012 Cal. Yr.	2013 Cal. Yr.
China	0	0	0	0	0	0	1	33	825	586	303	77
EU-27	0	31	37	18	0	24	10	7	40	61	72	90
Indonesia	0	0	0	0	0	1	0	9	7	0	1	2
Mexico	0	0	13	0	0	9	35	161	153	65	66	32
S. Korea	0	0	0	0	0	0	0	0	23	0	0	0
Taiwan	1	20	27	16	9	9	9	6	7	4	1	1
Thailand	0	0	6	7	0	1	0	41	18	22	42	42
U.S.A.	756	1075	1445	1361	1505	1542	1837	1468	1347	2337	2879	3060
Vietnam	0	0	0	0	0	1	0	26	47	15	57	68
Others	8	1	11	10	15	2	1	0	6	10	24	35
TOTAL	764.8	1126.7	1538.7	1412.2	1528.6	1589.4	1892.7	1751.5	2472.2	3100.4	3347.1	3409.9

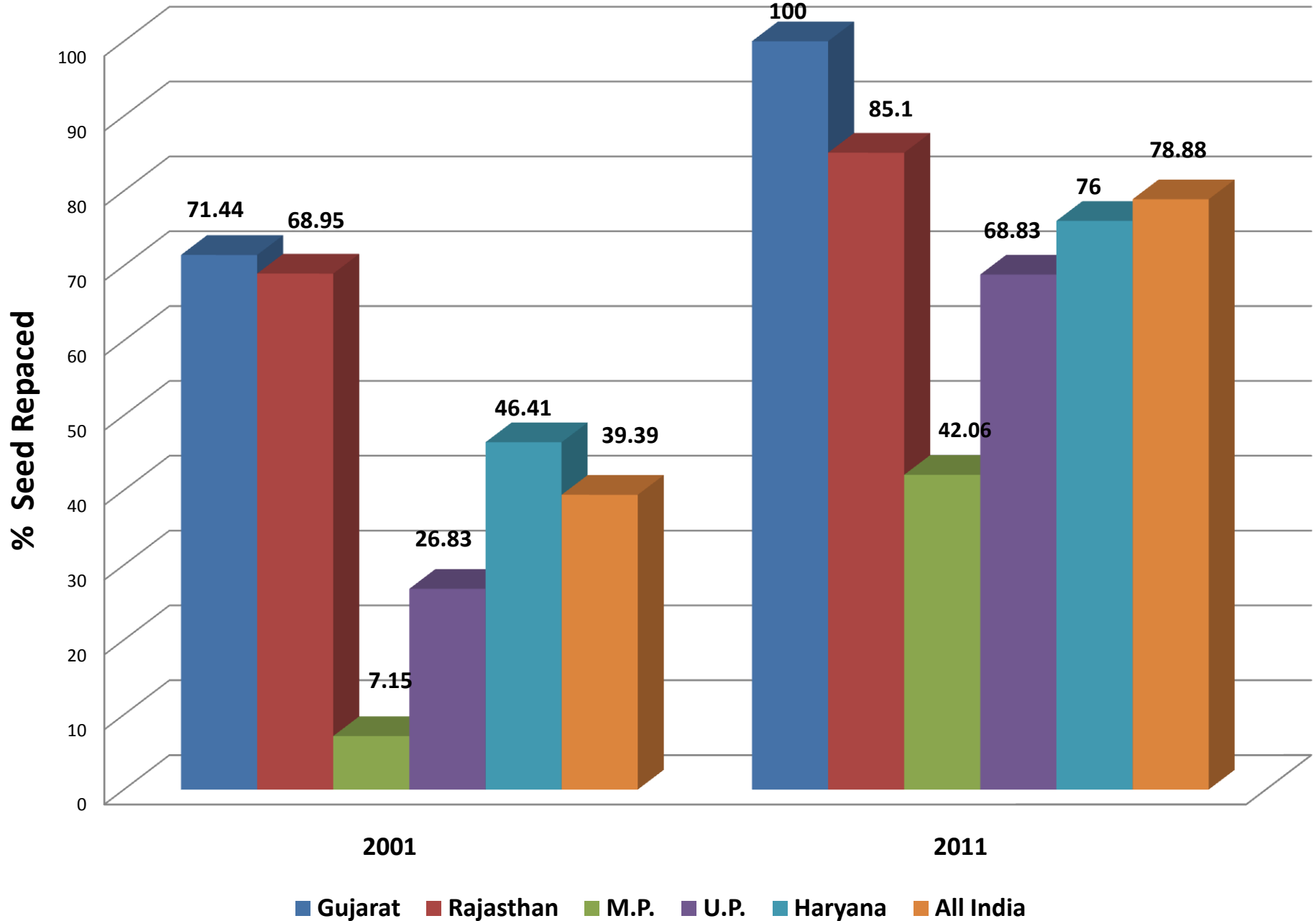
*Numbers may be off due to rounding.

Source: Canadian International Merchandise Trade Database - Statistics Canada

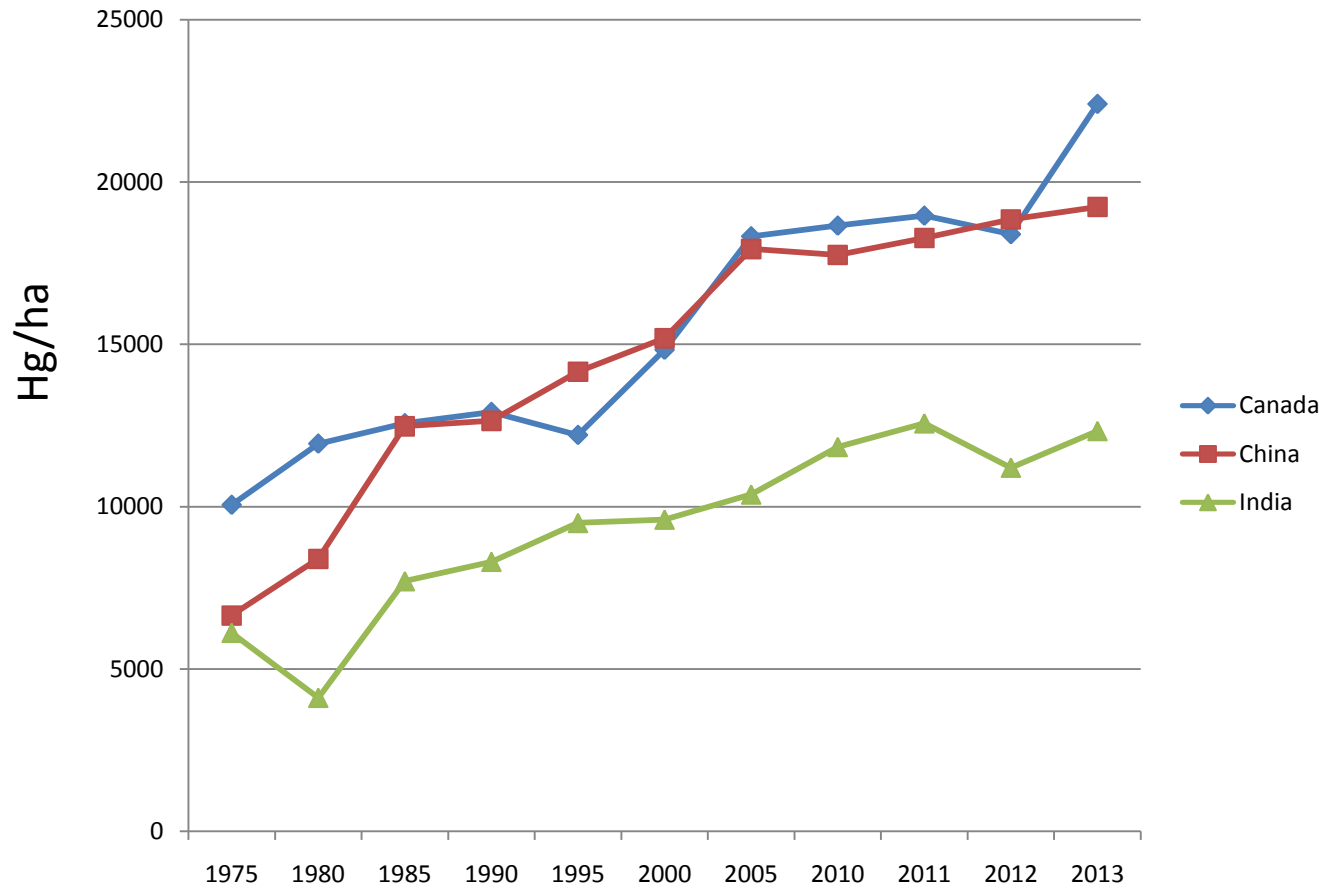
% Irrigated area under mustard



Seed replacement rate in mustard



Three country comparison of rapeseed mustard yield



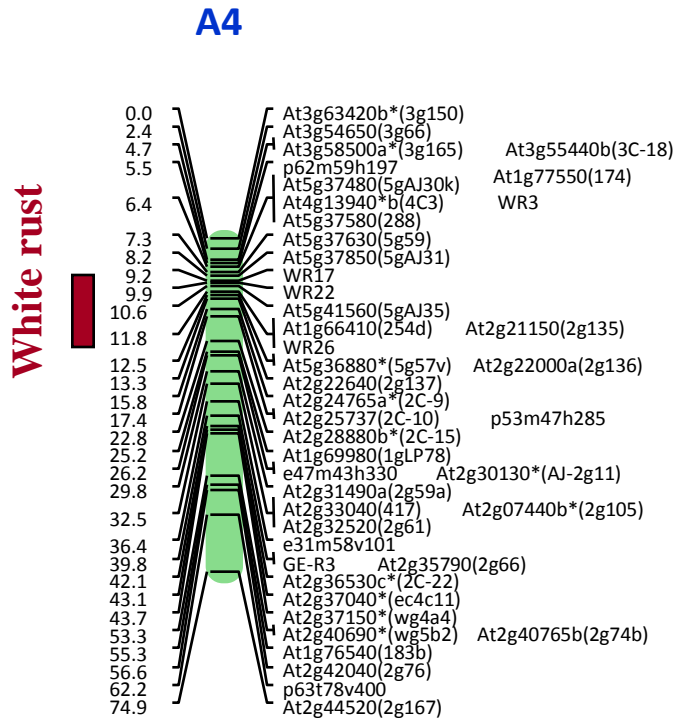
Domestic Production and Imports of Vegetable Oil

Source : Dept. of Agri and Dept. of Commerce, GOI

Year	Domestic Production MMT	Veg. Oil Imports MMT	Total Consumption MMT	Import Rs in Crore
1996-97	7.13	1.41	8.54	2929
1997-98	6.06	1.27	7.33	2765
1998-99	6.96	2.62	9.58	7589
1999-00	6.02	4.20	10.22	8046
2000-01	5.50	4.18	9.68	6093
2001-02	6.15	4.32	10.47	6465
2002-03	4.66	4.37	9.03	8780
2003-04	7.14	5.29	12.43	11683
2004-05	7.25	4.75	12.00	11077
2005-06	8.32	4.29	12.61	8961
2006-07	7.37	4.27	11.64	9540
2007-08	8.65	4.90	13.55	10301
2008-09	8.46	6.72	15.18	15838
2009-10	7.95	8.03	15.98	26483
2010-11	9.78	6.91	16.69	29860
2011-12	8.96	8.45	17.41	46255
2012-13	9.22	11.01	20.23	61107
2013-14	10.06	10.43	20.49	56776
2014-15	8.80	12.46	21.26	65184
2015-16				68927

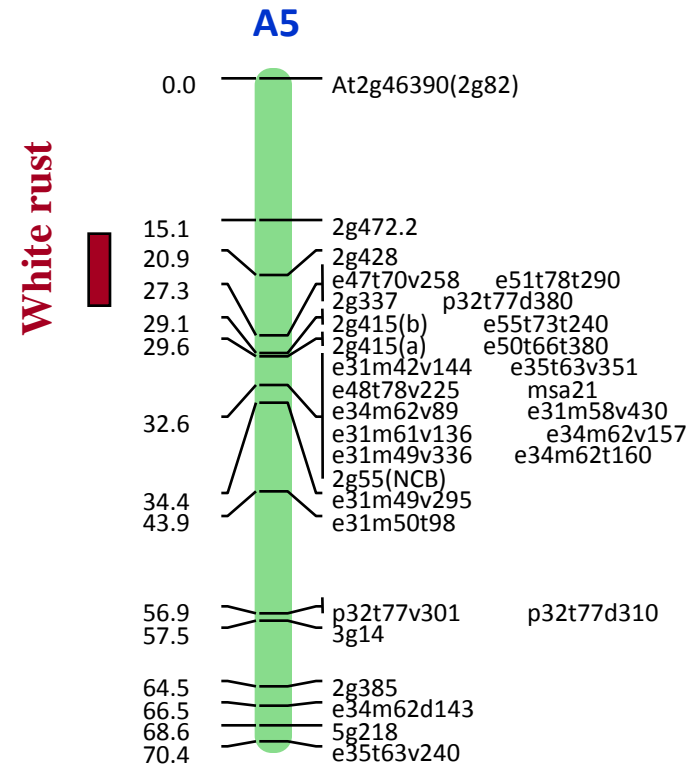
Two White rust resistance loci

Varuna-Heera



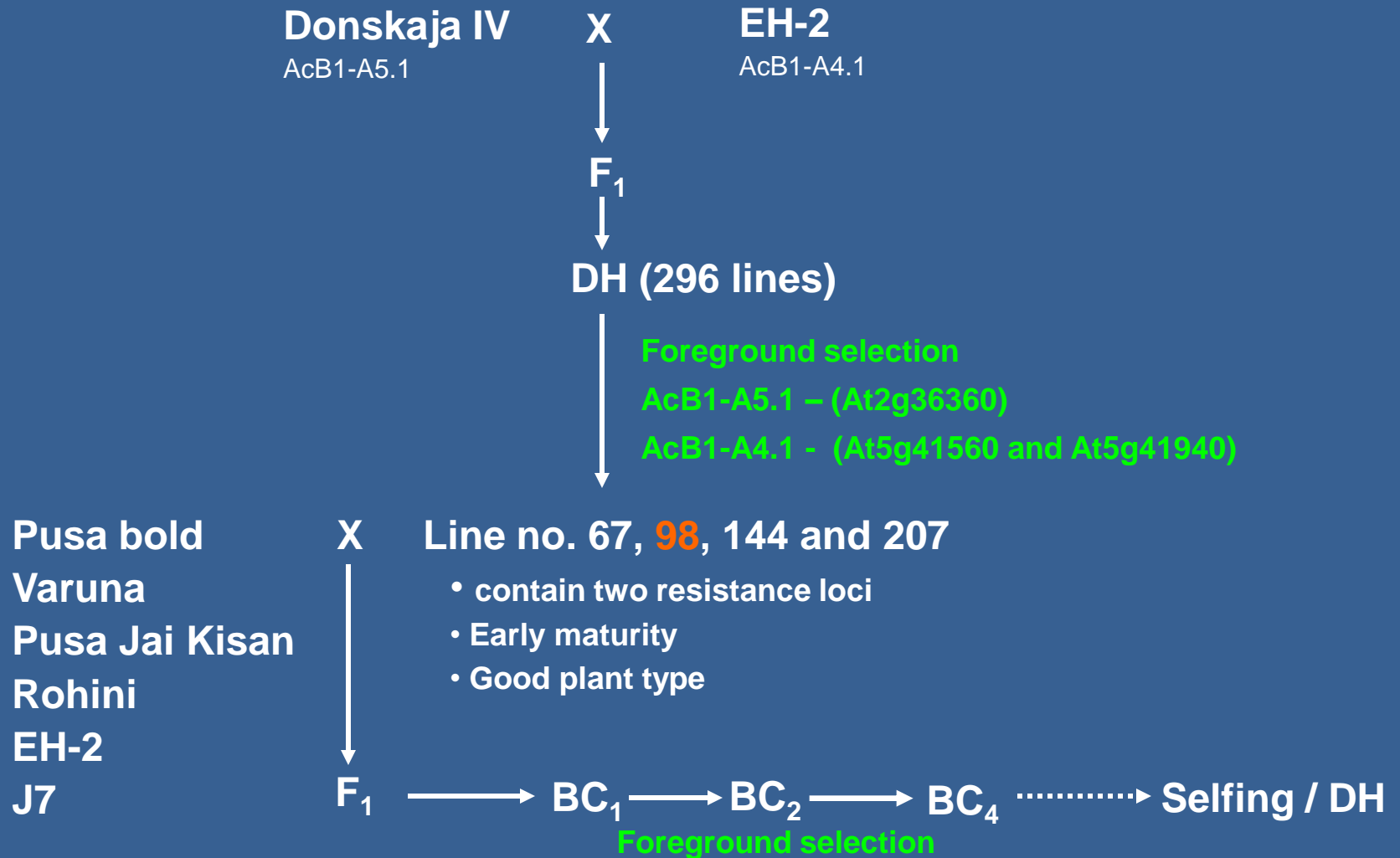
WR locus:7-17cM

TM4-Donskaja



WR locus:18-24cM

Marker-assisted introgression of two white rust resistance loci into popular Indian varieties of mustard



R & D Pipelines

Developed countries



India

