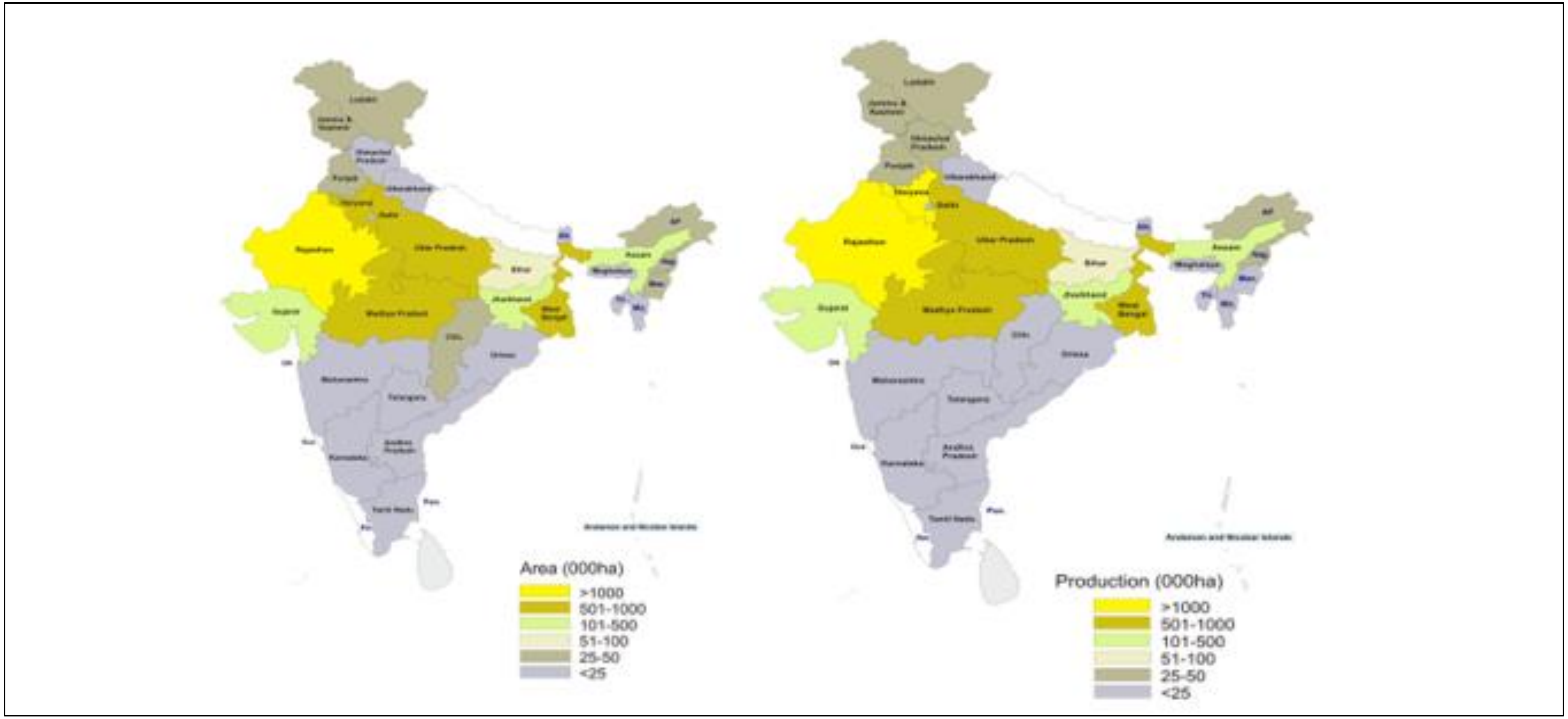
GENETIC GAIN IN BRASSICA BREEDING IN INDIA

SURINDER S BANGA

RAPESEED-MUSTARD : AREA AND PRODUCTION

India is the third largest producer of rapeseed-mustard after Canada and China. The total area under these crops in India during 2022-23 was estimated at 9.577 million hectares with a production of 11.5 million metric tons, which is almost 11 % of the world's total production.



RAPESEED-MUSTARD CROPS CULTIVATED IN INDIA

We grows five different kinds of oilseed brassicas, but *Brassica juncea* accounts for over 80% of the area under these crops.

Together these crops contribute over 23 % to total vegetable oil production of the country.

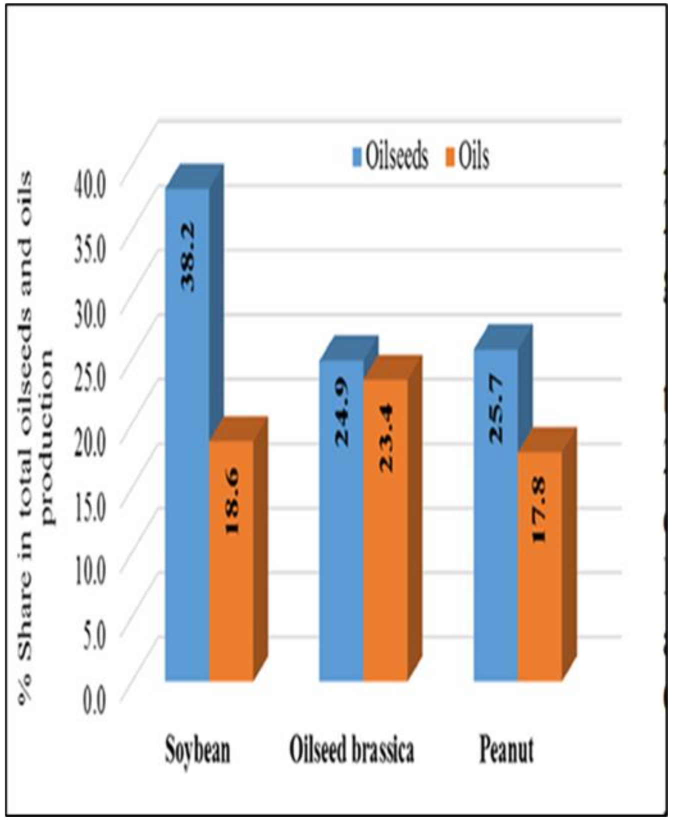
Brassica juncea

Brassica napus

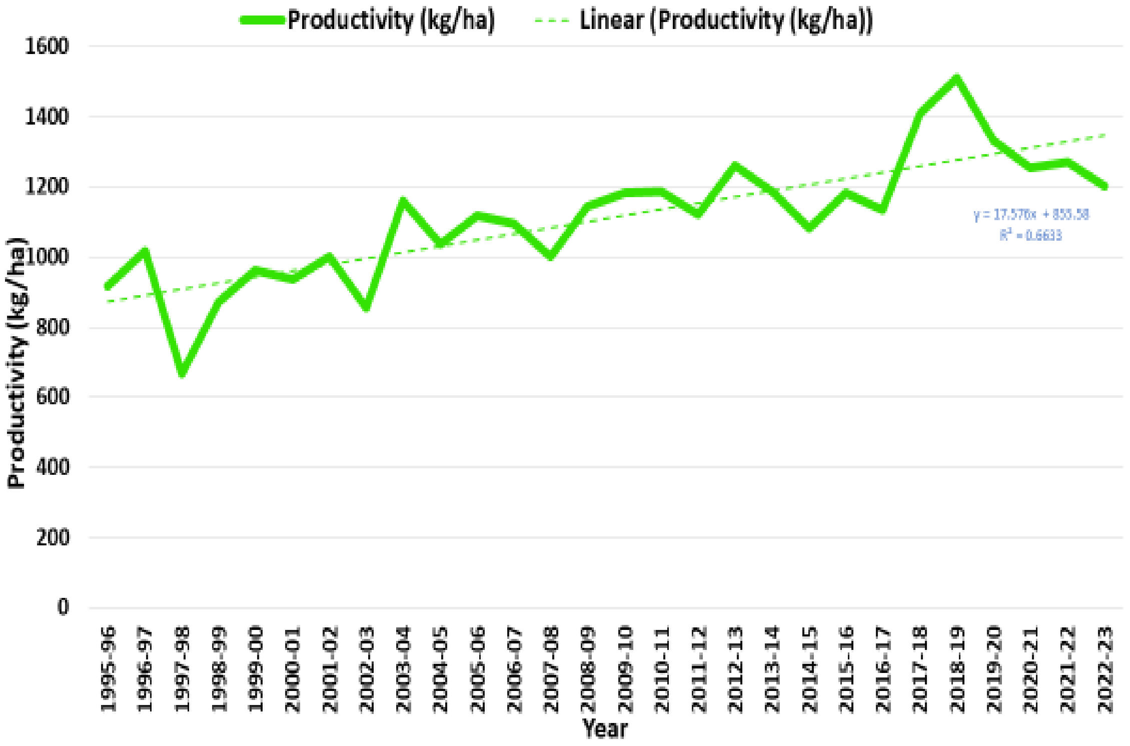
Brassica carinata

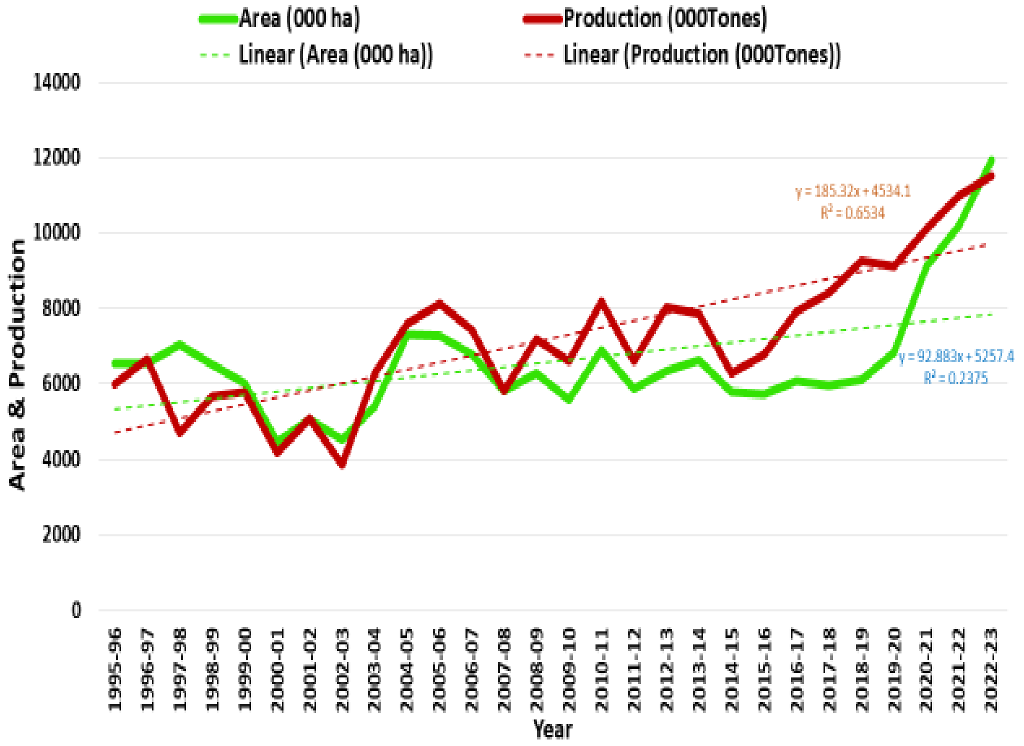
Brassica rapa Toria

Brassica rapa Yellow sarson



RAPESEED-MUSTARD CROPS IN INDIA: GROWTH TRAJECTORIES FOR PRODUCTIVITY AREA AND PRODUCTION





Productivity and production have grown at compound annual growth rates of 1.84% and 1.86%, respectively.

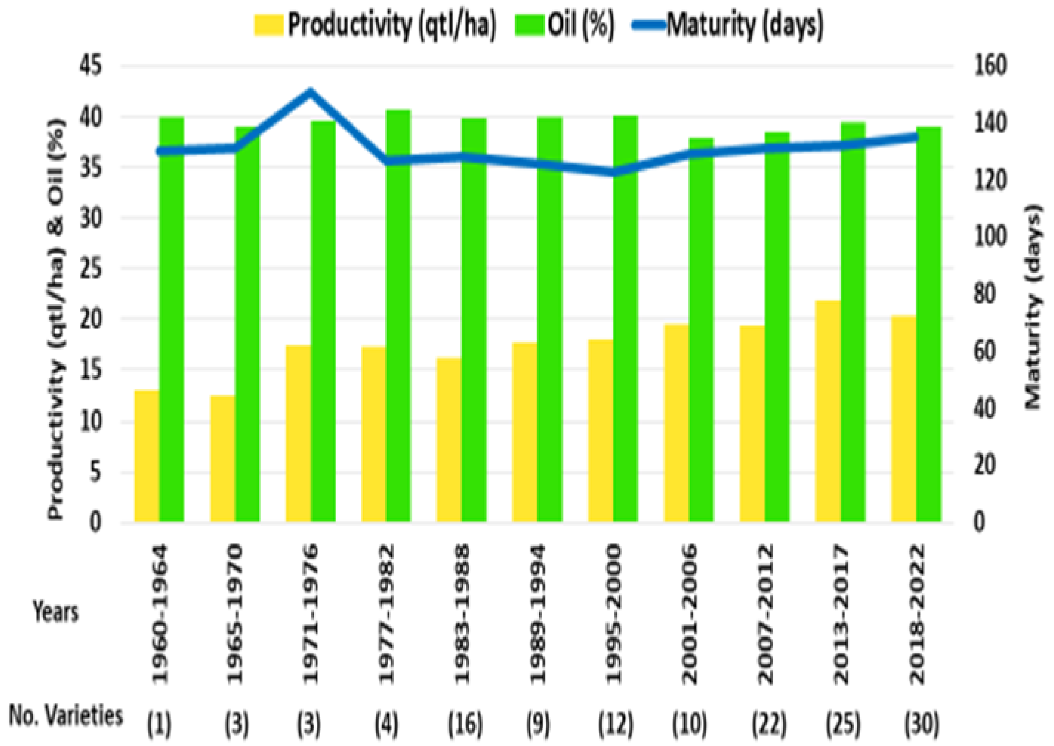
FOCUS TRAITS,BREEDING SYSTEMS AND BREEDING METHODS

- Rapeseed-mustard breeding is largely public funded , but private sector participation is increasing due to the success of mustard hybrids.
- Crop improvement efforts have largely aimed at improving yields, oil content, oil quality, and resistance to biotic and abiotic stresses.
- Breeding systems in rapeseed-mustard crops range from complete self-pollination to total cross-pollination.
- Pedigree and performance-based selection are mainly used to develop OP varieties in mustard canola rapeseed and yellow sarson.
- Mass selection and composite breeding are commonly followed in cross-pollinated toria; use of recurrent selection is rather rare.
- The availability of cytoplasmic male sterility and fertility restoration sources is heralding a switch over to the development of single cross hybrids in both mustard and canola rapeseed.

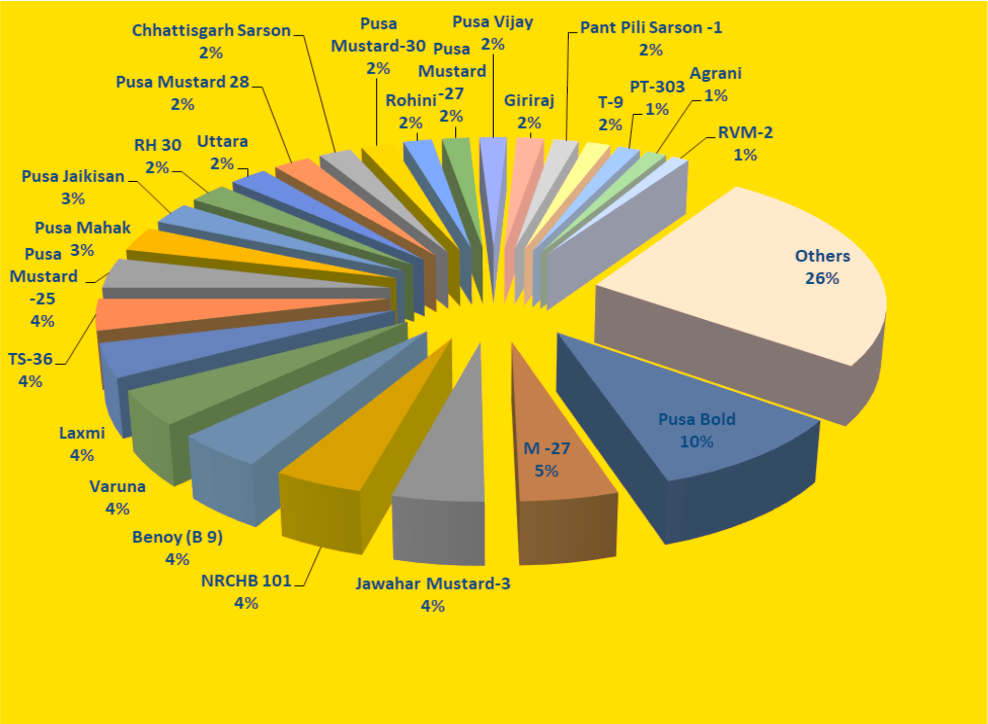
VARIETIES DEVELOPED AND BREEDING METHODS FOLLOWED FOR RAPESEED-MUSTARD IMPROVEMENT IN INDIA (1936-2022)

Period	Number of varieties	Selection	Hybridization	Induced mutation	Hybrids	Somaclone
1936-1966	26	26	-	-	-	-
1967-1970	03	03	-	-	-	-
1971-1980	21	15	04	02	-	-
1981-1990	41	13	22	03	-	-
1991-2000	40	13	22	02	02	01
2001-2010	72	16	47	05	03	-
2011-2022	58	8	44	02	04	-
Total	261	94	140	14	09	01

VARIETAL DEVELOPMENT IN *BRASSICA JUNCEA*



Over 261 varieties including nine hybrids have been released for rapeseed –mustard crops in India.



To 20 varieties account for almost 80% of total breeder seed indents in rapeseed-mustard crops (2010-11 to 2019-20)

IS THERE A SLOWDOWN IN PRODUCTIVITY GROWTH IN RAPESEED-MUSTARD?

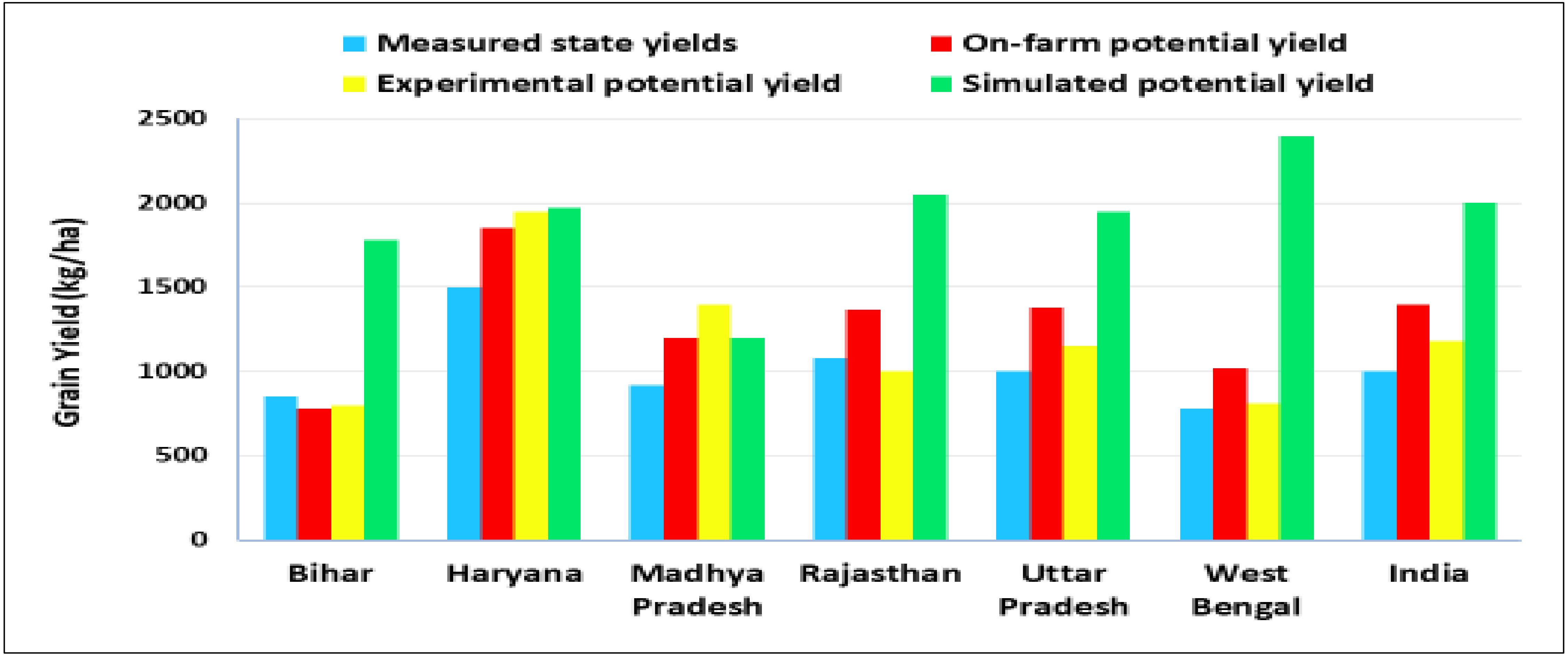
- Available data suggests that adoption of improved varieties has helped to steadily increase average productivity over the years. However, long-term trends also suggest some slowdown in productivity growth.

- The extent of genetic gains differs across regions in the country, which can be attributed to cropping intensity, available crop growth duration, agronomic practices followed, and environmental conditions.

- Productivity levels also show wide year to year fluctuations, which are largely due to the inability to secure rapeseed-mustard varieties against various biotic or abiotic stresses.

- The gap between potential yields and realized yields is still very large.

YIELD GAPS : COMPARISON BETWEEN THE MEASURED STATE AVERAGE MUSTARD YIELDS AND SIMULATED, EXPERIMENTAL, AND ON-FARM POTENTIAL YIELDS UNDER RAINFED CONDITIONS



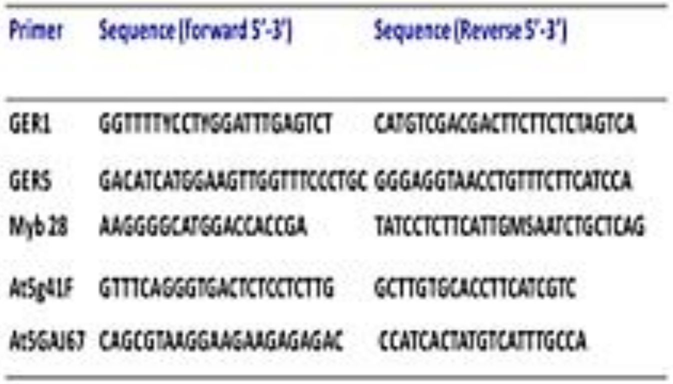
ENHANCING GENETIC GAINS

- **Analysis of foregoing slides indicate that domestication - or breeder-related genetic bottlenecks have reduced genetic diversity and possibly loss of rare alleles the in working germplasms.**
- **A broader shift to hybrids may promote greater use of genetic diversity and its classification into distinct heterotic pools.**
- **But, without the introduction of new alleles, existing genetic bottlenecks caused by inbreeding or targeted trait selection will continue to restrict genetic gains in both hybrids and OP varieties.**
- **Sustained yield gains require breeding innovations that optimize and expedite the response to selection.**
- **For that to happen, there is a need to aggressively catalogue, recombine, induce, or introduce novel genetic variations in into primary crop gene pools.**

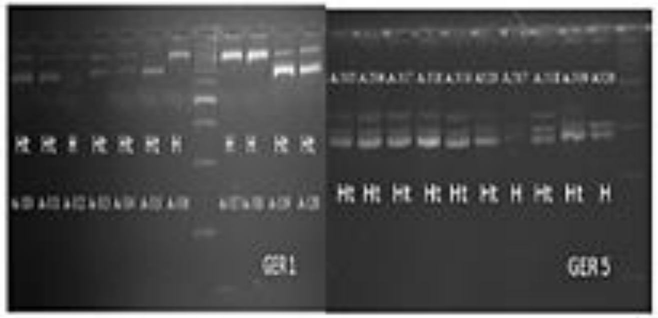
OPTIMISING BREEDING EFFICIENCES THROUGH MARKER ASSISTED BREEDING

Increasing use of KASPar markers for erucic acid and gene-based markers for meal glucosinolates, oleic acid, and white rust resistance promises faster breeding gains for seed quality modifications as compared to traditional phenotypic selection.

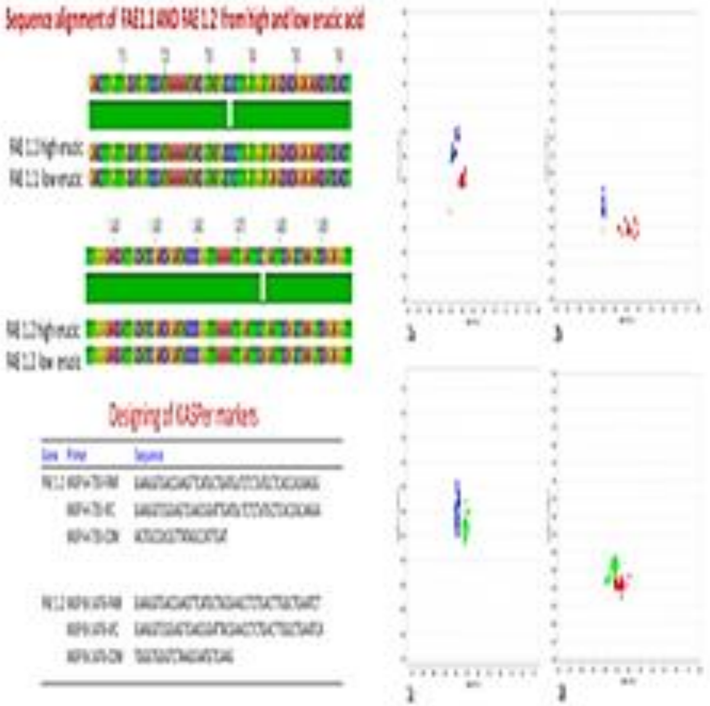
GENE BASED MARKERS FOR GLUCOSINOLATES



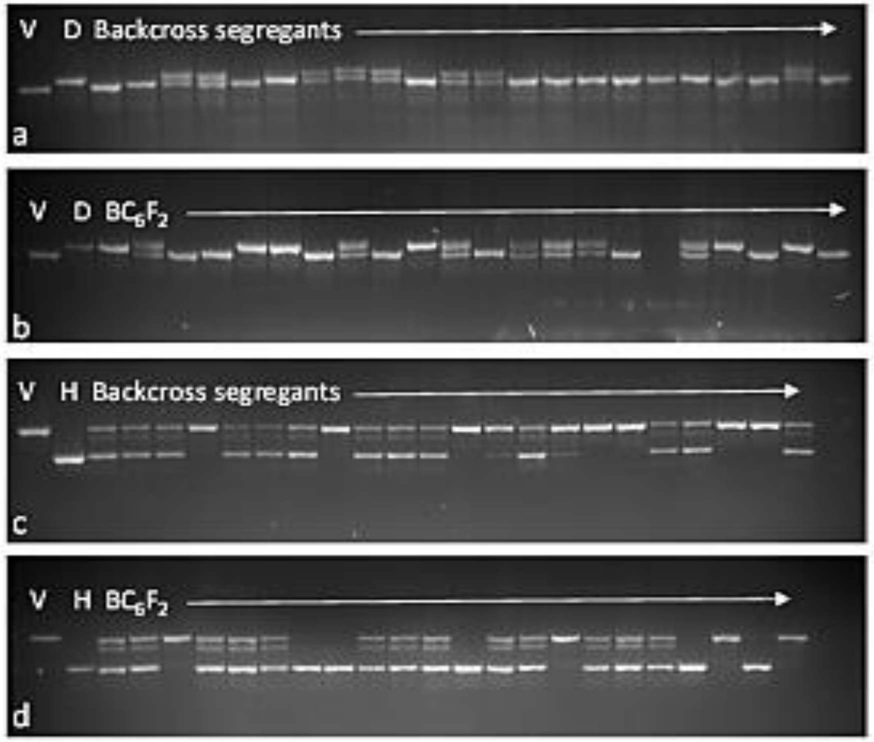
Primer	Sequence (Forward 5'-3')	Sequence (Reverse 5'-3')
GER1	GGTTTTYCCTYGGATTTGAGTCT	CATGTCGACGACTTCTTCTCTAGTCA
GER5	GACATCATGGAAGTTGGTTTCCCTGC	GGGAGGTAACCTGTTTCTTCATCCA
Myb 28	AAGGGGCATGGACCACCGA	TATCCTCTTCATTGMSAATCTGCTCAG
At5g41F	GTTTCAGGGTGACTCTCCTCTTG	GCTTGTGCACCTTCATCGTC
At5GAI67	CAGCGTAAGGAAGAAGAGAGAC	CCATCACTATGTCATTTGCCA



KASP MARKERS FOR LOW ERUCIC ACID



MARKER GENOTYPING DNA PROFILES OF *ACB1-A5.1* AND *ACB1-A4.1*



HYBRID BRASSICAS

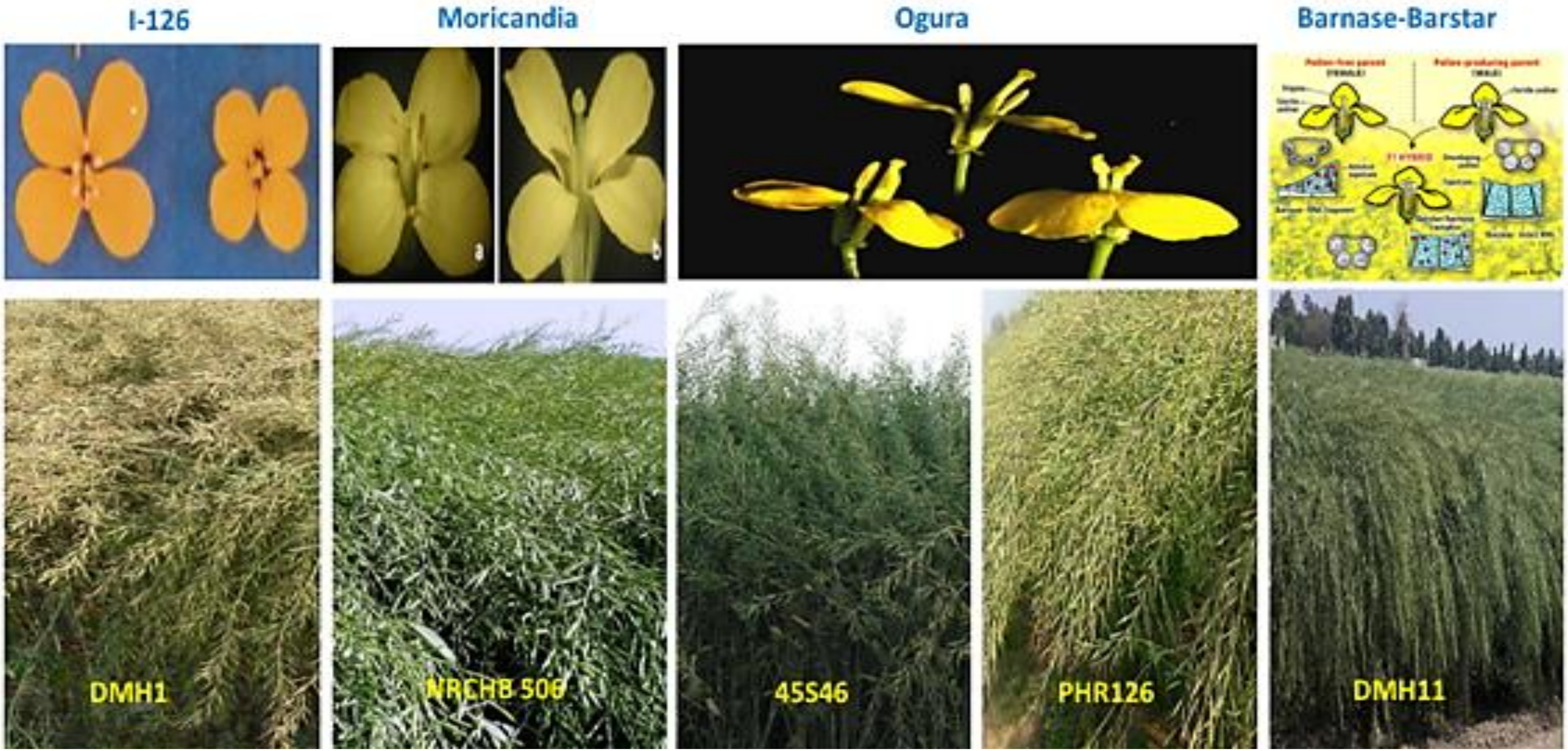
A large number of CMS-Rf systems have been developed and at least three of these have been used commercially. Ogura CMS is the most commonly used.

Heterosis over the best inbred(s) is high under space planting, but it is generally <15 % at commercial sowing densities.

Inbreeding depression is very low in large field plots, and the use of F_2 seeds is very common among the farmers, without obvious yield losses.

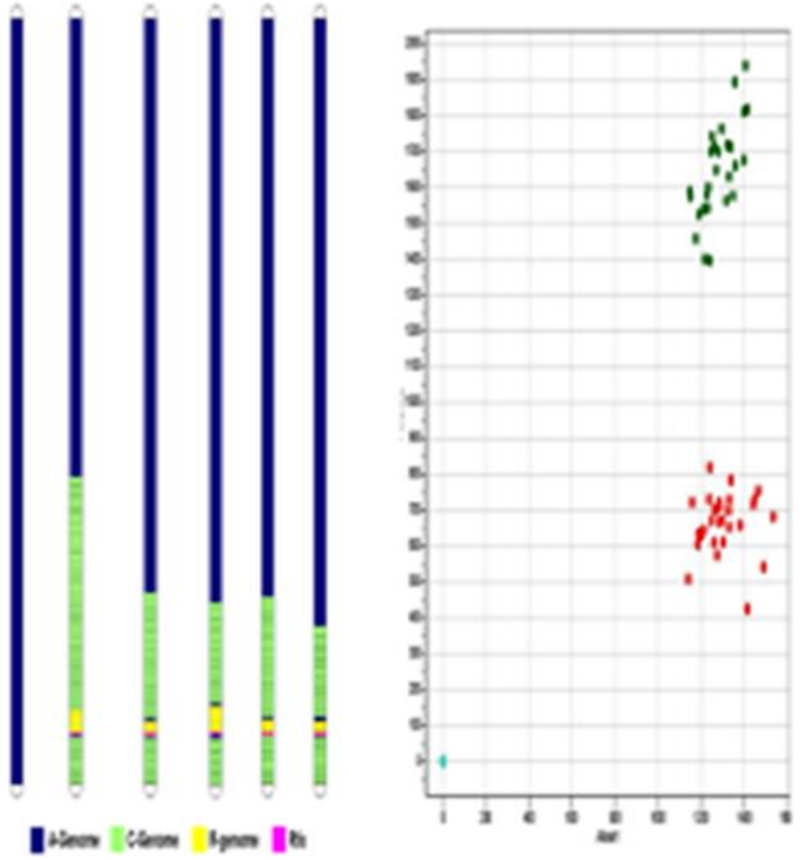
Heterotic gene pools are still to be adequately defined, and natural crop gene pools are all mixed up.

POLLINATION CONTROL SYSTEMS AND HYBRID BREEDING IN *BRASSICA JUNCEA*

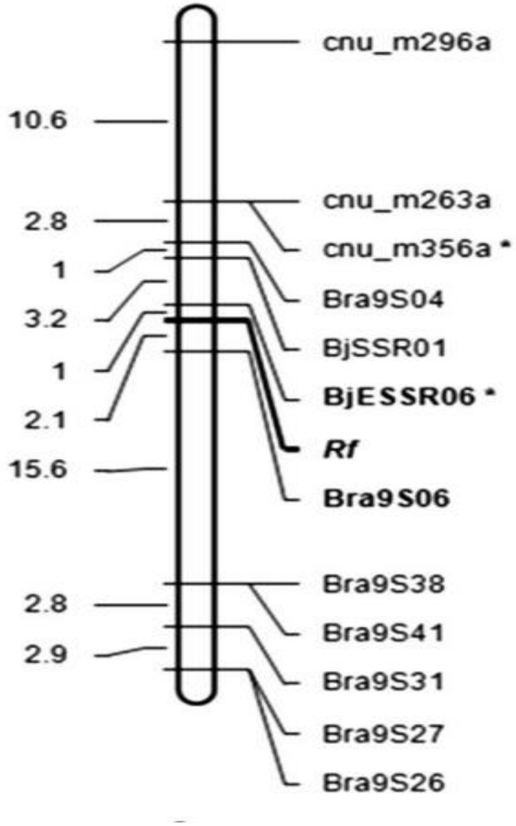


HYBRID MUSTARD : MAPPING RF GENES FOR KEY CMS SYSTEMS

Mapping gene for fertility restoration for *ogu* CMS and a KASP marker



Mapping gene for fertility restoration for *Moricandia* CMS



SEED QUALITY MODIFICATIONS

- Oil quality has been greatly improved by nearly removing erucic and eicosenoic acids in local genotypes of both *B.juncea* and *B.napus.*
- Meal glucosinolate levels were also brought down to <30 micromoles per gm. defatted meal.
- A number of OP varieties and one “00” hybrid have been released for cultivation in mustard.
- Three OP varieties and three Canola *B.napus* hybrids are under cultivation, primarily in cooler areas of north-west India. Canola GSC 7 is a very popular in canola *B. napus* growing areas.

“00” MUSTARD HYBRID RCH 1

CANOLA NAPUS GSC 7

CANOLA NAPUS HYBRID PHSH1707

HIGH OLEIC MUSTARD

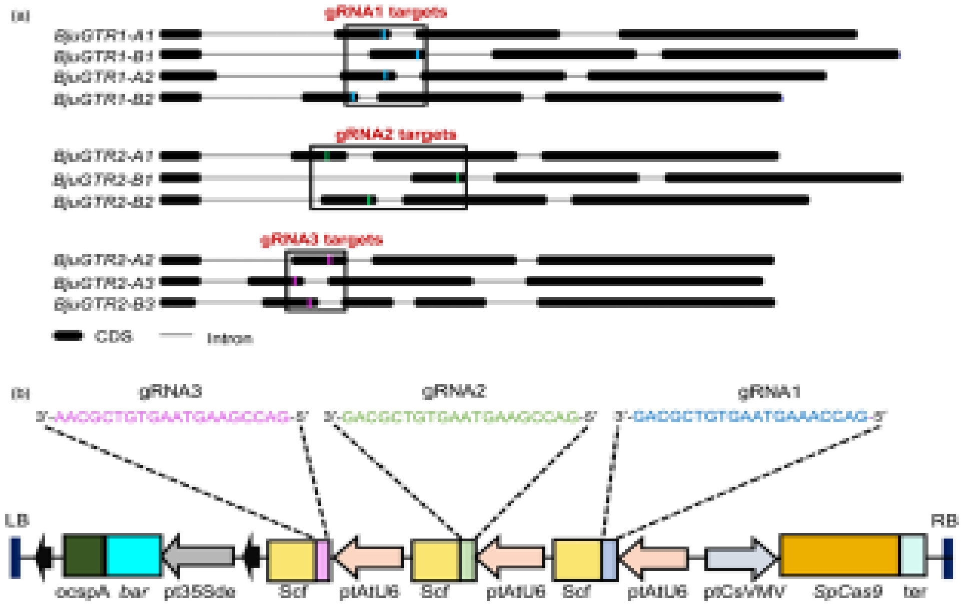
Current “00” mustard varieties possess low oleic acid(40-45%). Efforts are underway to enhance it to canola *B.napus* levels through breeding and genetic modifications.

- **High oleic (72%) and low linoleic acid (8%) transgenics have been by antisense suppression of the *FAD2* gene.**

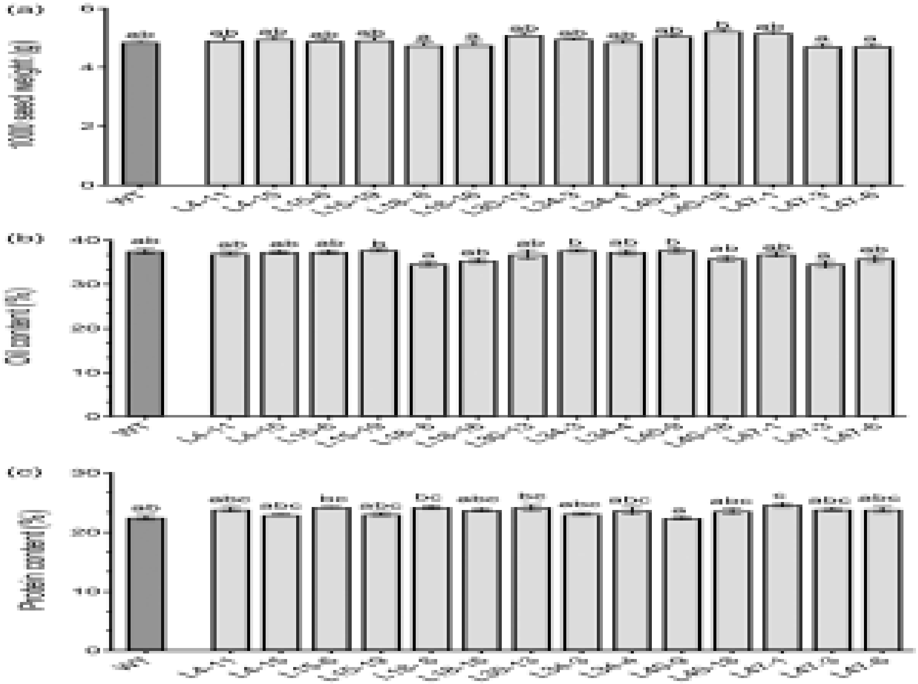
- **Genotypes with intermediate oleic acid (≈60%), have been bred through C-genome introgression in low erucic acid mustard. Marker development underway.**

- **A low erucic acid genotype (low erucic allele of *FAE1.1+FAE1.2* deletion) is being used to create extra-high oleic acid mustard by combining it with a frame-shift deletion in the B genome orthologue of *FAD2.C5* and a potential mis-sense mutation in the A genome orthologue of *FAD2.C5* (In collaboration with Prof. Ian Bancroft).**

TARGETTED EDITING OF GTR1 and GTR 2 HOMOLOGS FOR THE DEVELOPMENT OF LOW SEED AND HIGH LEAF GLUCOSINOLATE MUSTARD



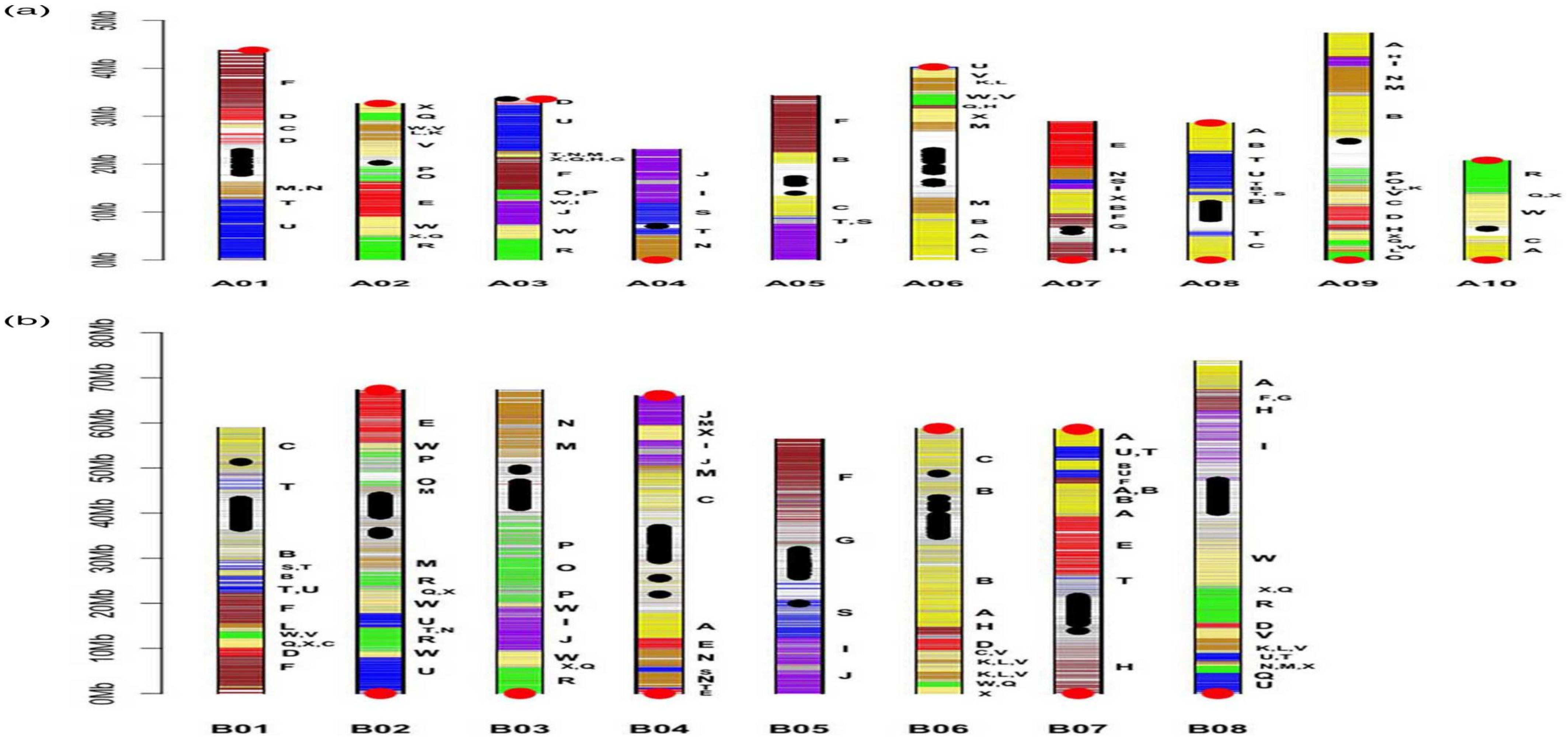
Targeted editing of multiple homologues of GTR1 and GTR2 genes



Ideal low-seed, high-leaf glucosinolate oilseed mustard with uncompromised defence and yield

Plant Biotechnology Journal, DOI: (10.1111/pbi.14121)

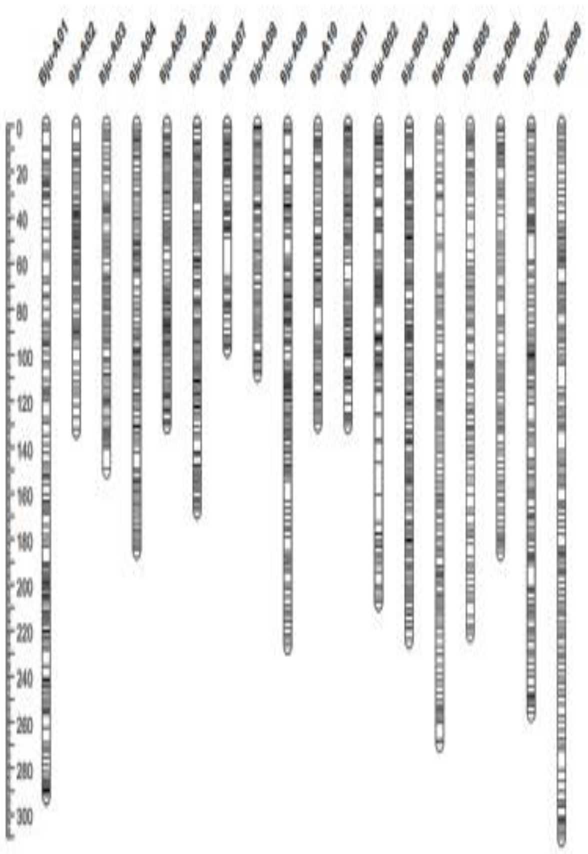
TOWARDS GENOME ASSISTED BREEDING: A CHROMOSOME-SCALE ASSEMBLY OF ALLOTETRAPLOID *BRASSICA JUNCEA* CV. VARUNA



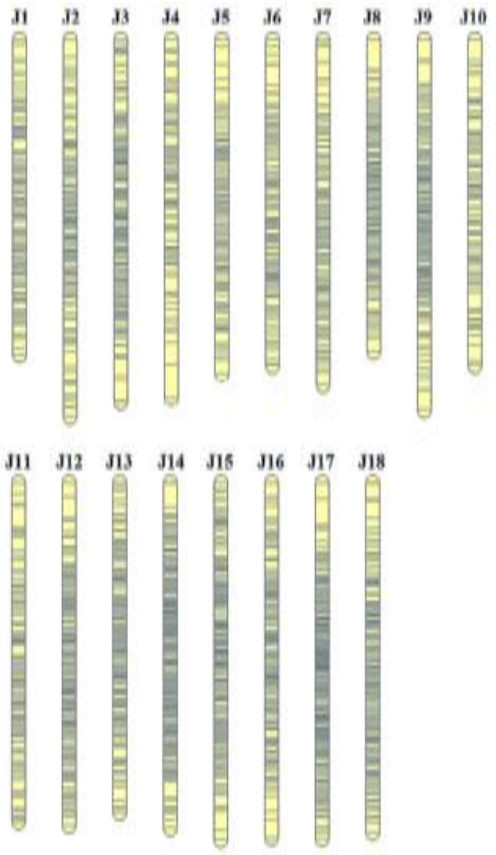
Plant Biotechnology Journal, Volume: 19, Issue: 3, Pages: 602-614, First published: 19 October 2020, DOI: (10.1111/pbi.13492)

HIGH DENSITY LINKAGE MAPS FOR MUSTARD

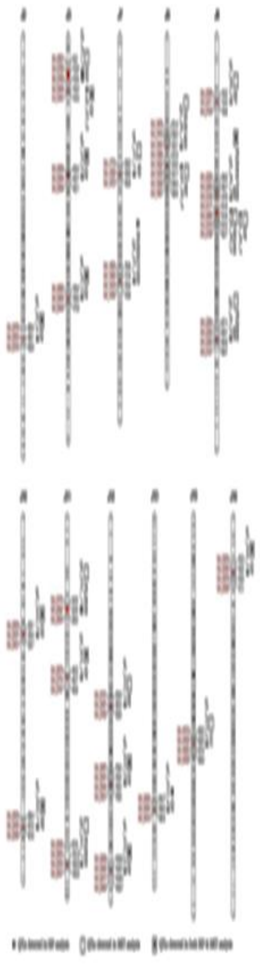
QTL MAPPING FOR FLOWERING,PRODUCTIVITY RELATED TRAITS AND SEED OIL CONYENT

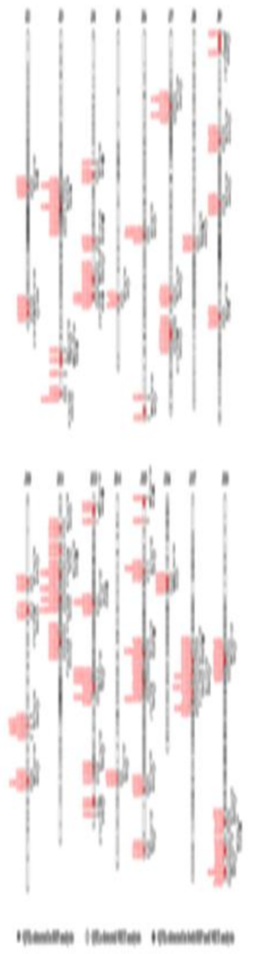


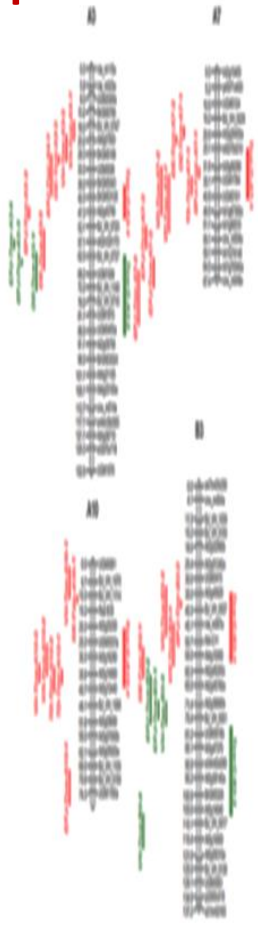
Brassica juncea Tumida x Varuna F1DH



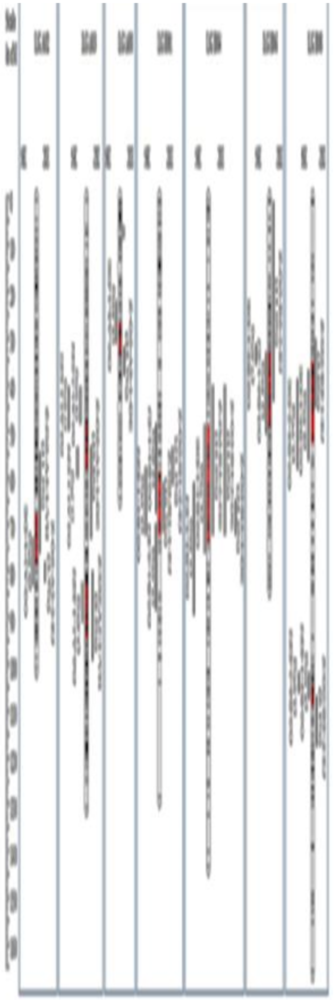
Natural x Resynthesized *B.juncea RIL*







Dhaka et al.2016



Rout et al.(2018)

BRASSICA JUNCEA DIVERSITY SETS : GWAS AND ASSOCIATIVE TRANSCRIPTOMICS

GWAS are now increasingly being used to identify genes underlaying trait variation.

Two large diversity sets (NASF panel and PORI panel) have been phenotyped across several locations in India.

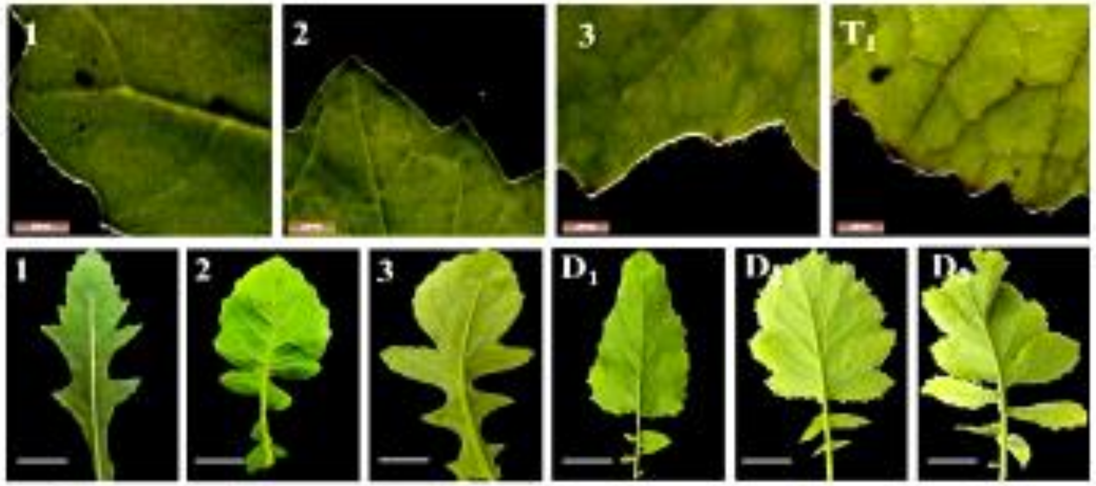
Genomic and expressed SNPs have been used to identify many haplotype blocks that are significantly correlated with quantitative trait variation.

INTROGRESSED RESISTANCE

Wild donors	Recipient species species	Target trait
BIOTIC STRESSES		
Diplotaxis erucoides *Sinapis alba*	*B.juncea*	Alternaria blight
Erucastrum cardaminoides, Diplotaxis tenuisiliqua and *B.fruticulosa*	*B. napus* *B. juncea*	Sclerotinia rot
B. fruticulosa	*B. juncea*	Aphid
ABIOTIC STRESSES		
Erucastrum abyssinicum	*B. juncea*	Cold/frost Heat and drought

Alternaria blight disease responses in BC_1F_2 and BC_2 progenies derived from *B.juncea* + *Sinapis alba* somatic hybrid.

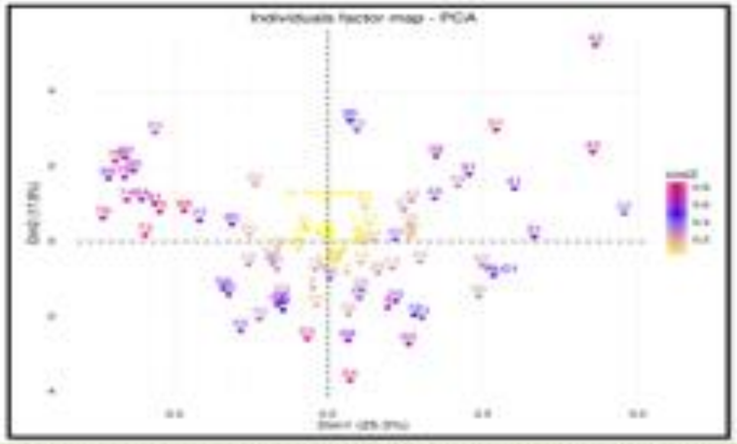
Alternaria resistance in *B.juncea-Diplotaxis erucoides* ILs



INTROGRESSED RESISTANCE TO SCLEROTINIA STEM ROT

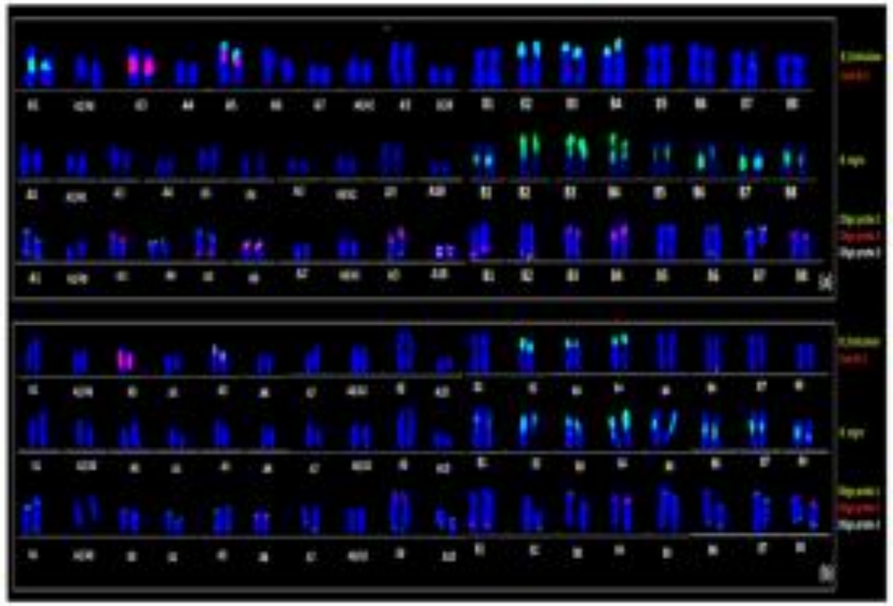
SV and RNA-seq GWAS based on the pan genome developed using *B. juncea-B. fructiculosa* ILs have allowed the prediction of 38 candidate genes. Over 11 of these could be partially validated for their role in introgressed resistance to the sclerotinia pathogen.

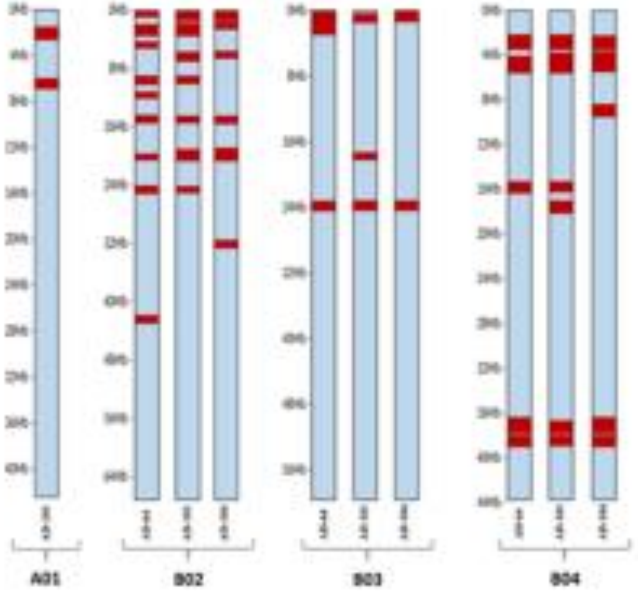
RESISTANCE TO MUSTARD APHID IN *B.JUNCEA* INTROGRESSED FROM *B.FRUTICULOSA*



variate analysis based on phenotypic traits (AII, APP and PPI) and defensive (POD, CAT, NADH oxidase, PPO, H_2O_2 and Total phenols)

uticulosa and select introgression lines (yellow) expressed resistance against *L.* *ui* infestation, depicted by low phenotypic and high defensive trait values





RESISTANCE TO ABIOTIC STRESSES

Frost damage

Heat stress

Drought damage

Donors: 1. *Erucastrum abyssinicum*
2. *Diplotaxis tenuisiliqua*

Developed Brassica juncea-Erucastrum abyssinicum and *B.juncea- Diplotaxis tenuisiliqua* introgression lines which vary for resistance to these stresses

UTILIZATION OF INTROGRESSED VARIATION FOR CULTIVAR DEVELOPMENT

- **Alien introgressions for resistance to mustard aphid, alternaria blight, and sclerotinia stem rot have been developed, but linkage drag continue to restrict their deployment in productive cultivars.**

- **Attempts to remove linkage drag have so far failed despite repeated recombination cycles.**

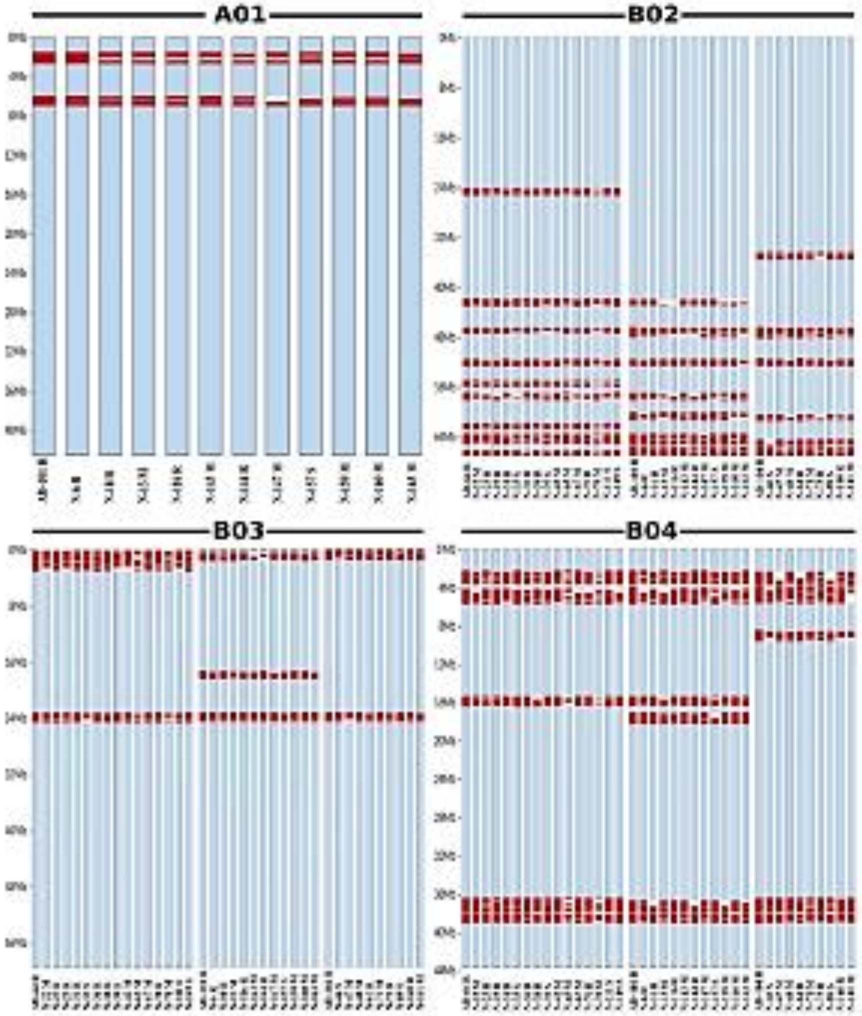
- **In some cases the linkage drag was recessive, only detectable in the homozygous state, making it impossible to select for phenotype based recombinants.**

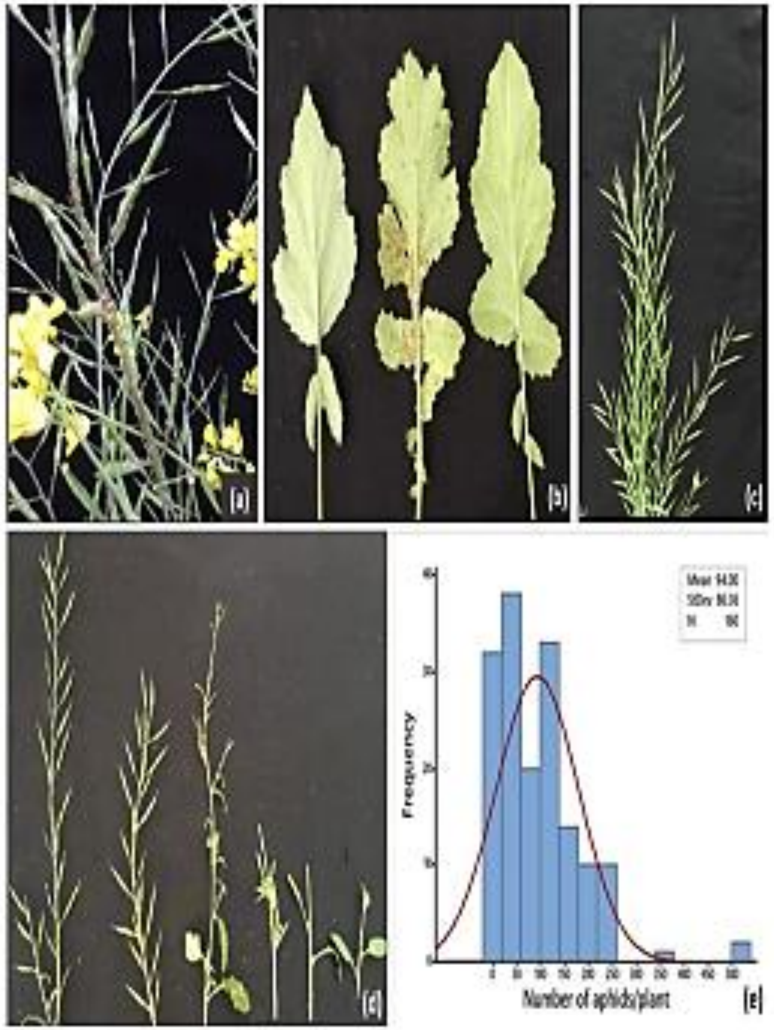
- **It may be possible to break linkage drag through physical disruption of introgressed alien fragments using very heavy doses of gamma irradiations.**

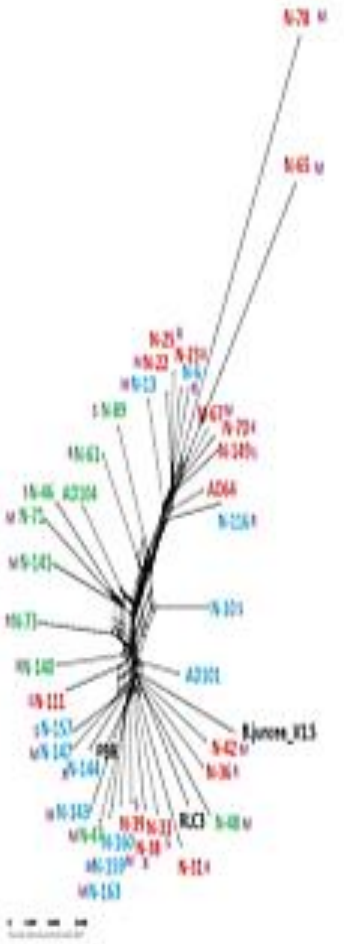
OR

- **More precisely through CRISPR aided selective chromosome segment deletions.**

DISRUPTION OF ALIEN SEGMENT SUBSTITUTIONS FOLLOWING GAMMA IRRADIATIONS IN *BRASSICA JUNCEA* ILs

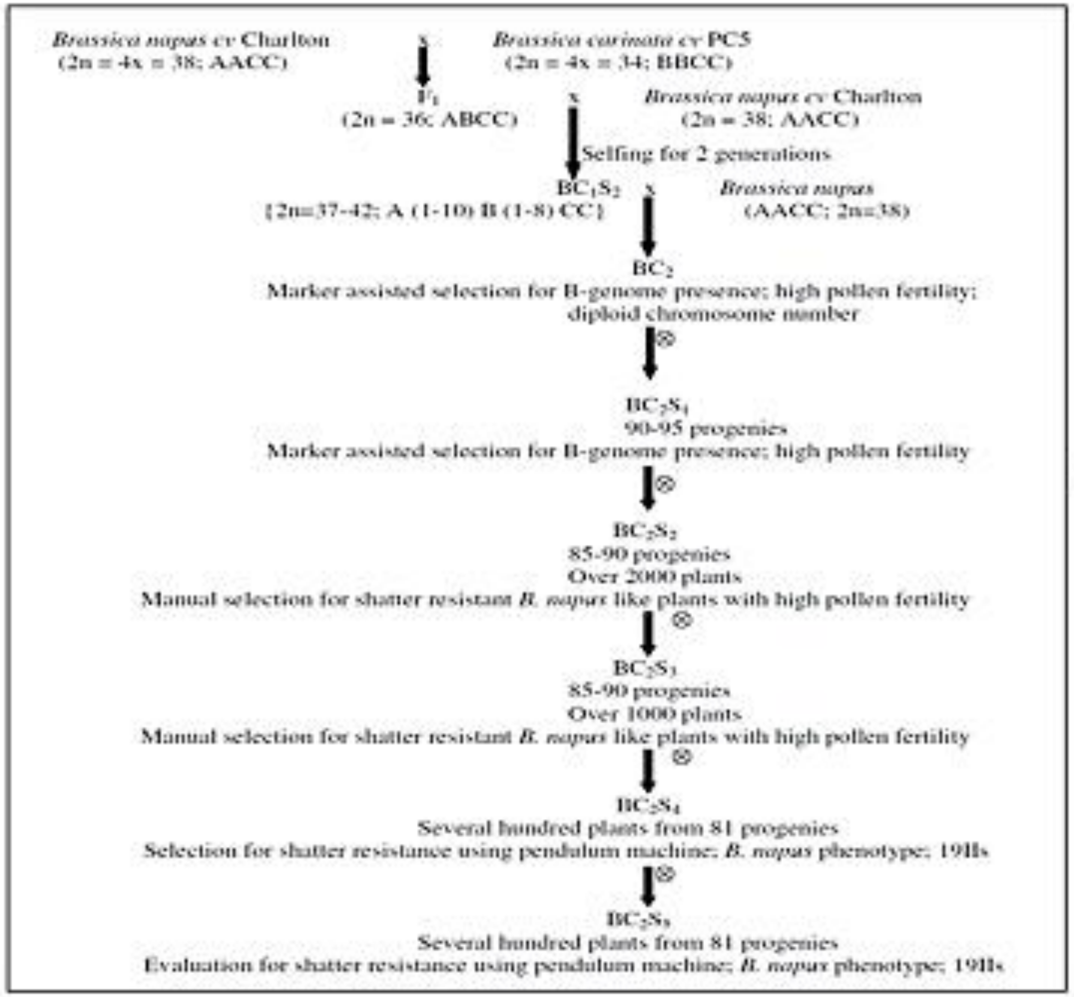






INTERNATIONAL COLLABORATIONS: GRDC, ACIAR , INDO- AUSTRALIA BIOTECHNOLOGY FUND & INDO-UK PORI PROJECT

INTROGRESSING GENOMIC FRAGMENTS FROM *B.CARINATA* TO *B.NAPUS*



Brassica napus cv Charlton (2n = 4x = 38; AACC) x *Brassica carinata cv* PC5 (2n = 4x = 34; BBCC)

F$_1$ (2n = 36; ABCC) x *Brassica napus cv* Charlton (2n = 38; AACC)

Selfing for 2 generations

BC$_1$S$_2$ {2n=37-42; A (1-10) B (1-8) CC} x *Brassica napus* (AACC; 2n=38)

BC$_2$
Marker assisted selection for B-genome presence; high pollen fertility; diploid chromosome number

⊗

BC$_2$S$_1$
90-95 progenies
Marker assisted selection for B-genome presence; high pollen fertility

⊗

BC$_2$S$_2$
85-90 progenies
Over 2000 plants
Manual selection for shatter resistant *B. napus* like plants with high pollen fertility

⊗

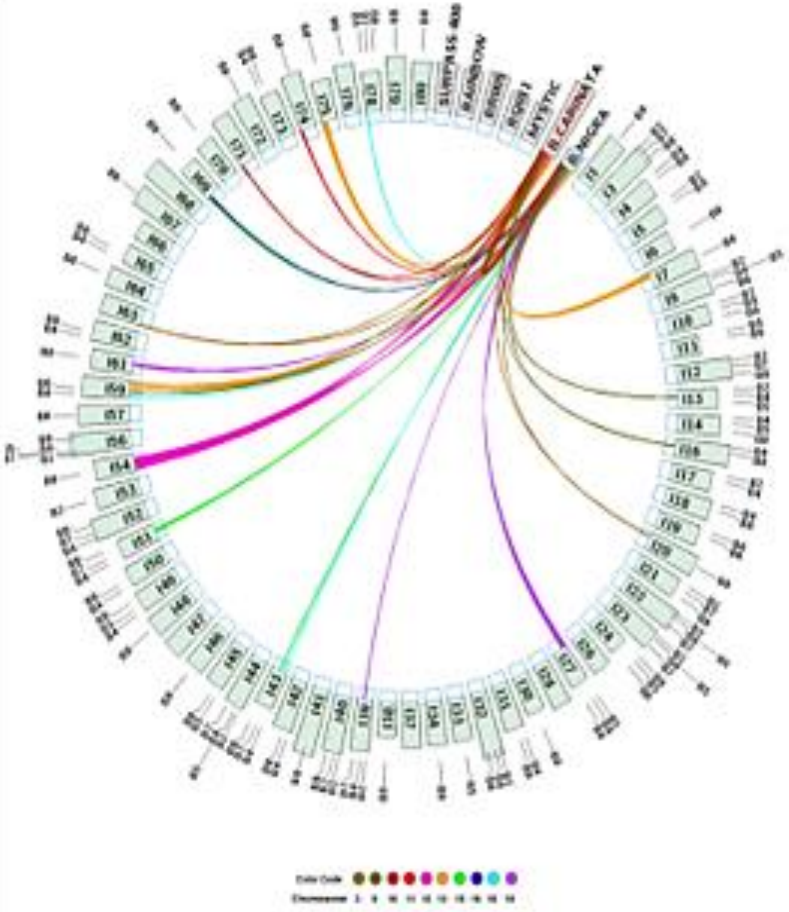
BC$_2$S$_3$
85-90 progenies
Over 1000 plants
Manual selection for shatter resistant *B. napus* like plants with high pollen fertility

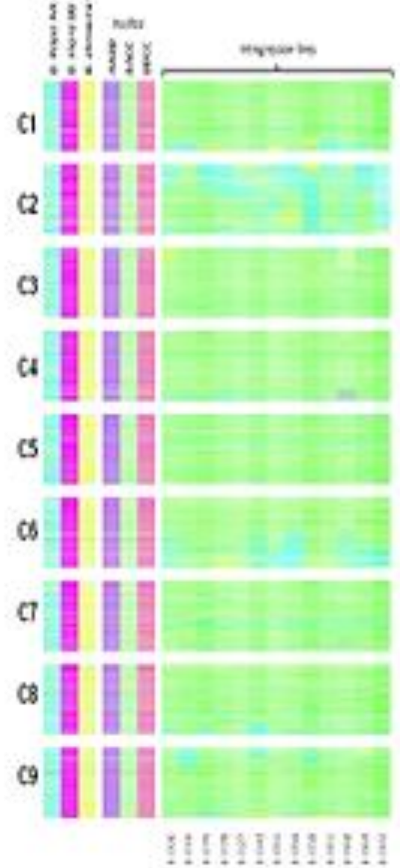
⊗

BC$_2$S$_4$
Several hundred plants from 81 progenies
Selection for shatter resistance using pendulum machine; *B. napus* phenotype; 19IIs

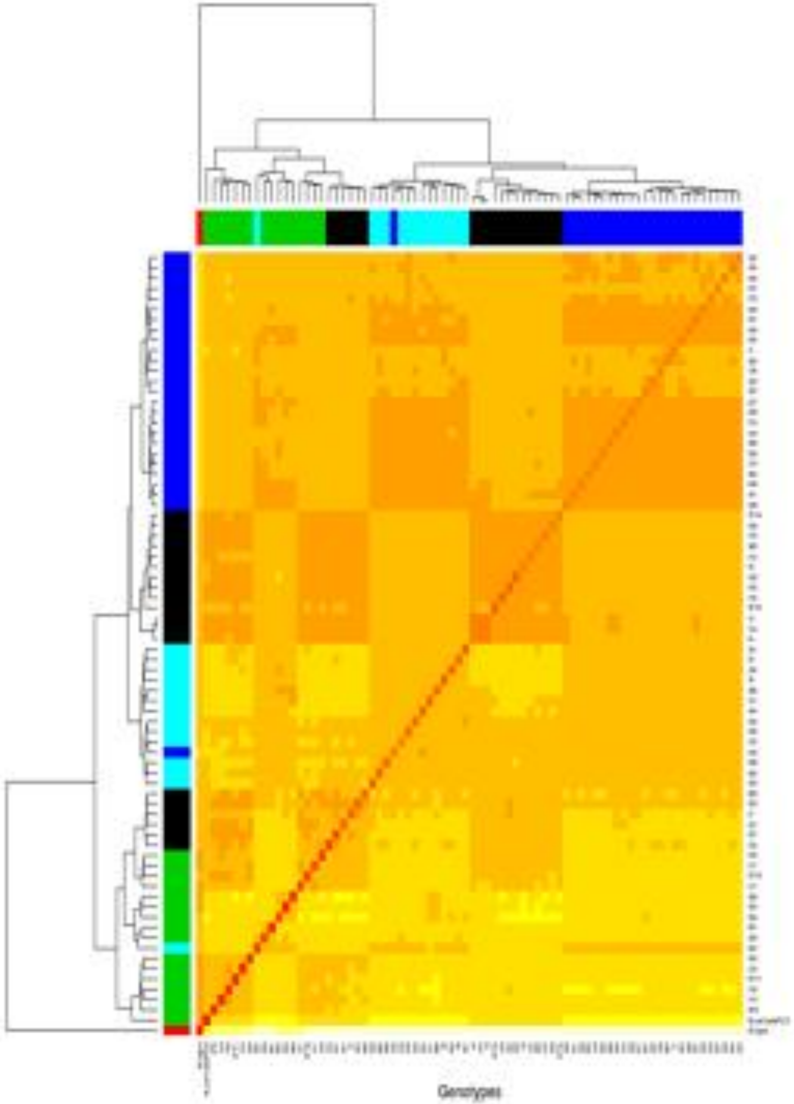
⊗

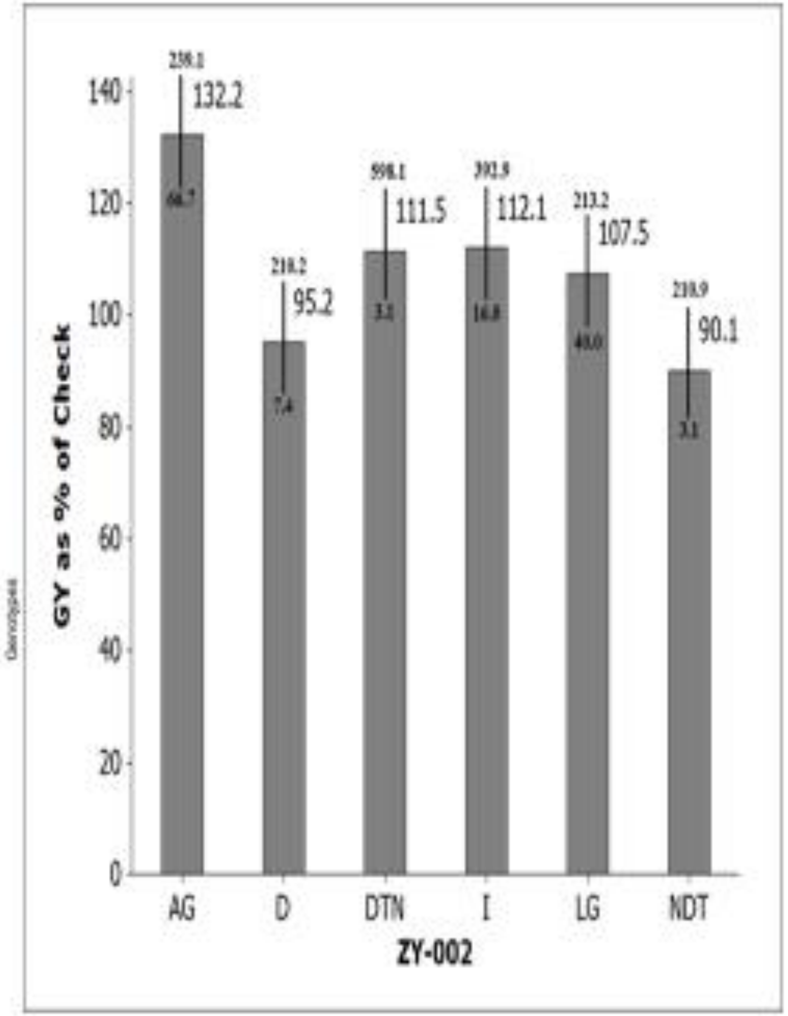
BC$_2$S$_5$
Several hundred plants from 81 progenies
Evaluation for shatter resistance using pendulum machine; *B. napus* phenotype; 19IIs



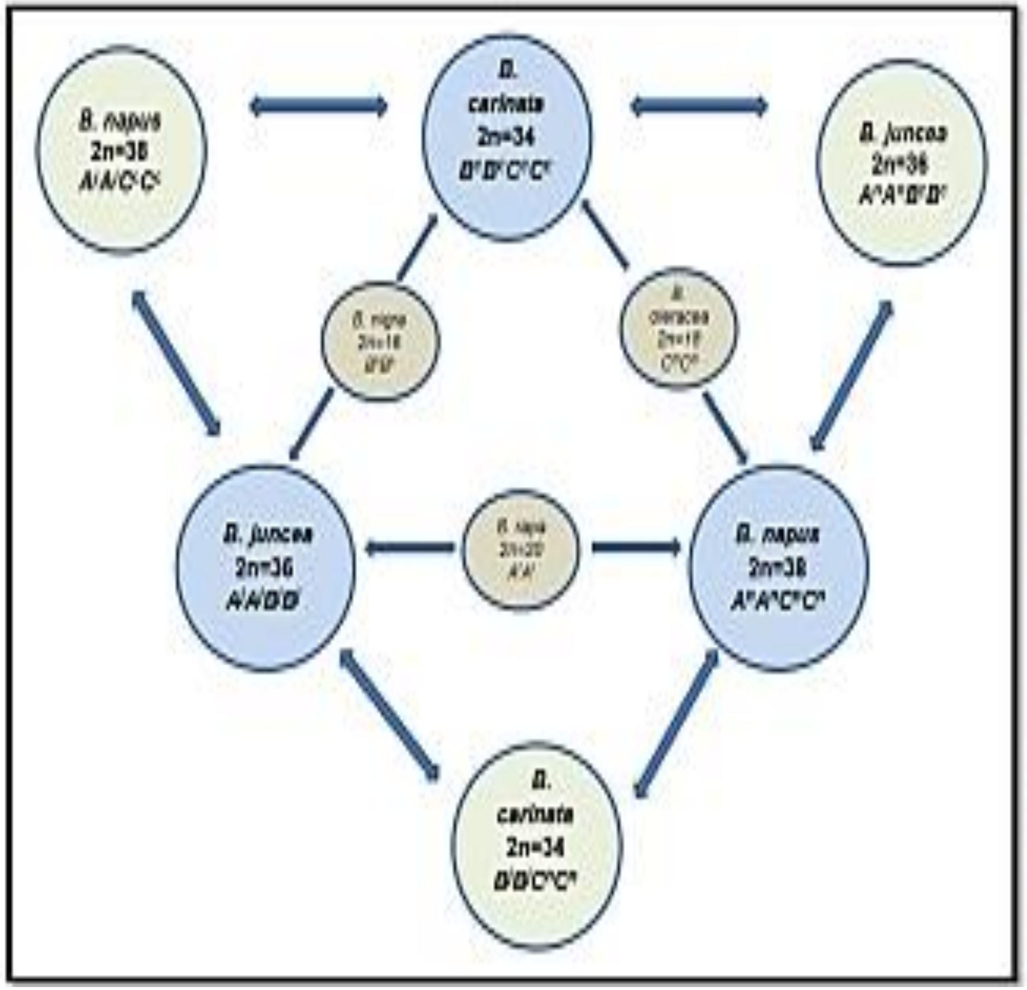


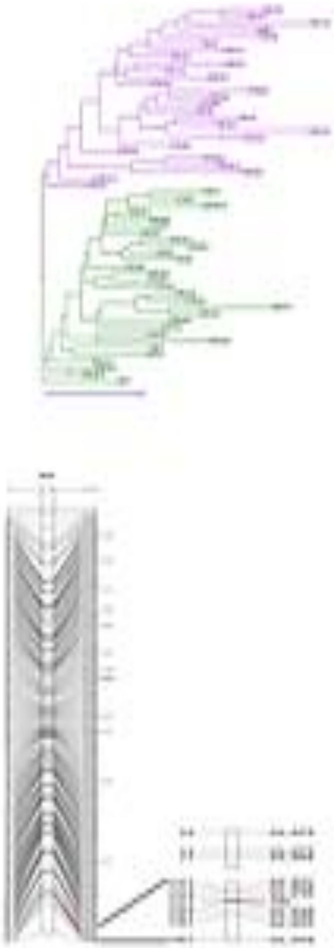
ENHANCING GENETIC DIVERSITY , HETEROSIS AND REDUCED POD SHATTERING THROUGH SYNTHESIS OF *B.NAPUS-B.CARINATA* INTROGRESSION LINES



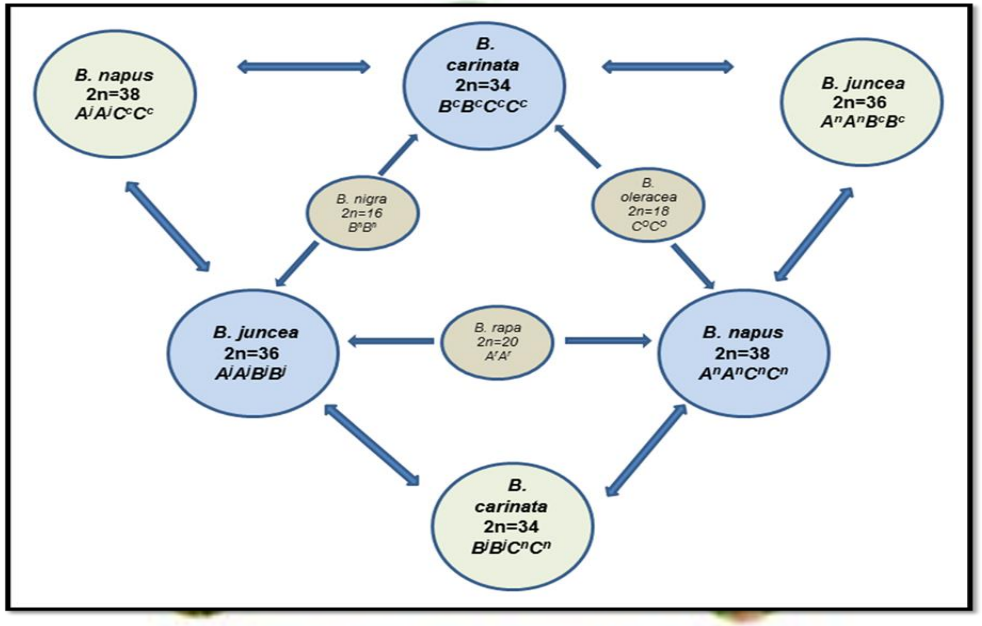


RESYNTHESIS OF *BRASSICA JUNCEA* AND C GENOME SEGMENT SUBSTITUTIONS





RESYNTHESIS OF *BRASSICA NAPUS* AND B GENOME SEGMENT SUBSTITUTIONS IN DERIVED FORMS



B. napus
2n=38
$A^jA^jC^cC^c$

B. carinata
2n=34
$B^cB^cC^cC^c$

B. juncea
2n=36
$A^nA^nB^cB^c$

B. nigra
2n=16
B^nB^n

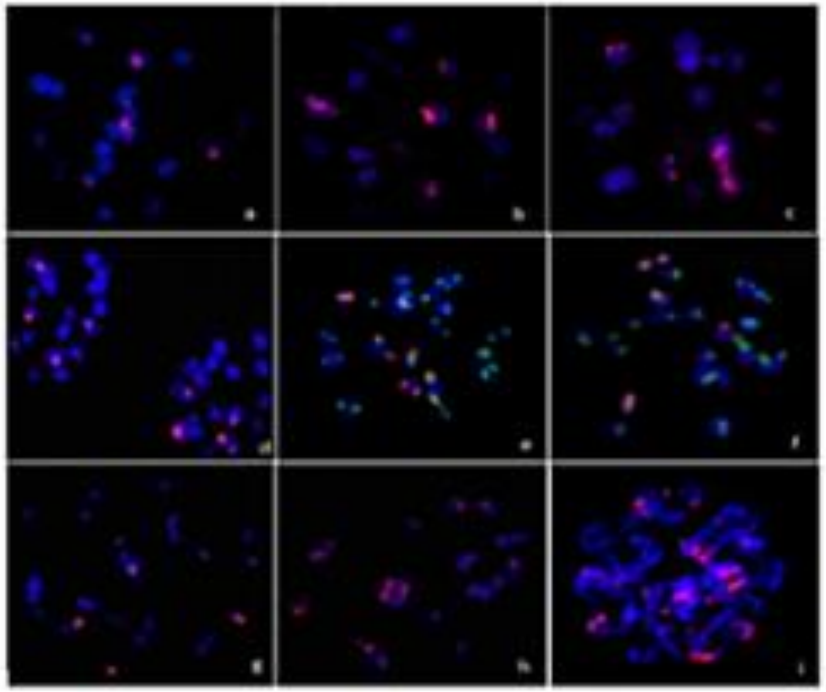
B. oleracea
2n=18
C^oC^o

B. juncea
2n=36
$A^jA^jB^jB^j$

B. rapa
2n=20
A^rA^r

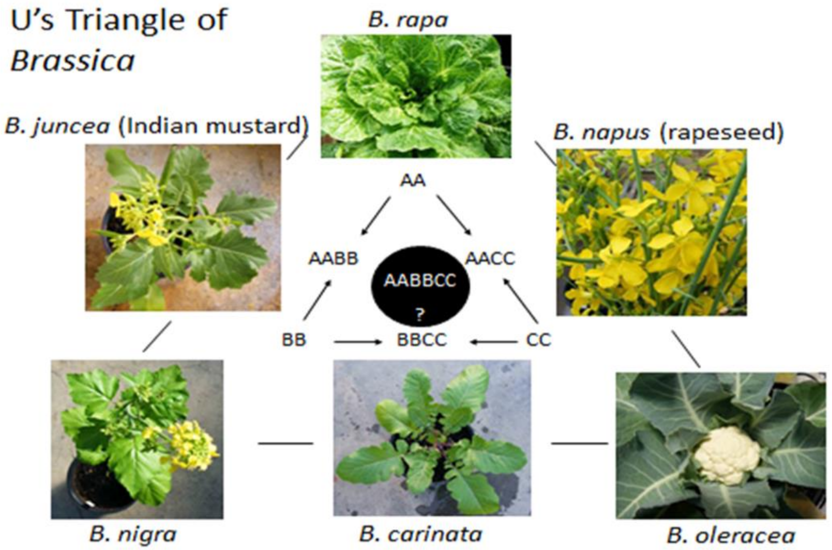
B. napus
2n=38
$A^nA^nC^nC^n$

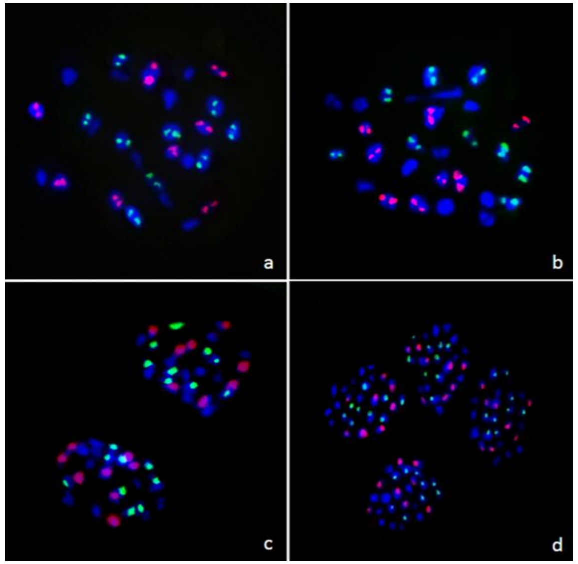
B. carinata
2n=34
$B^jB^jC^nC^n$

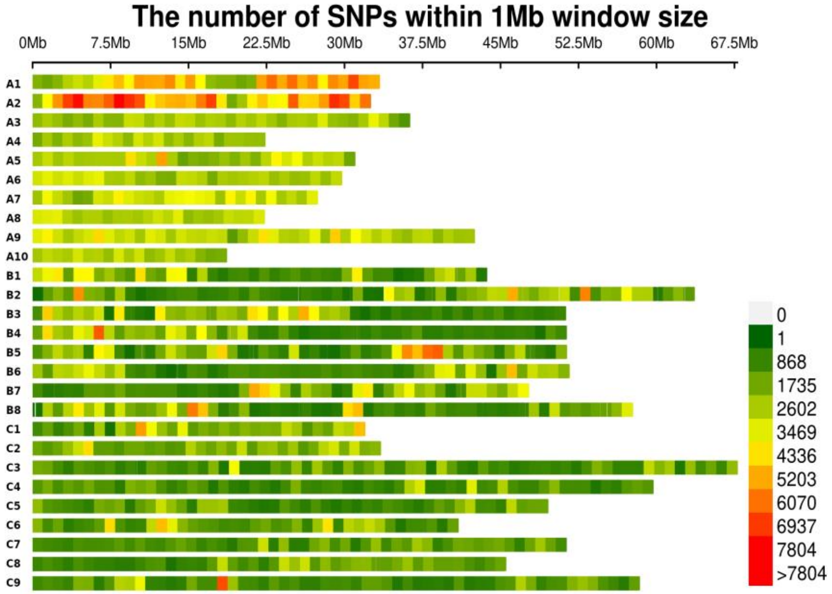


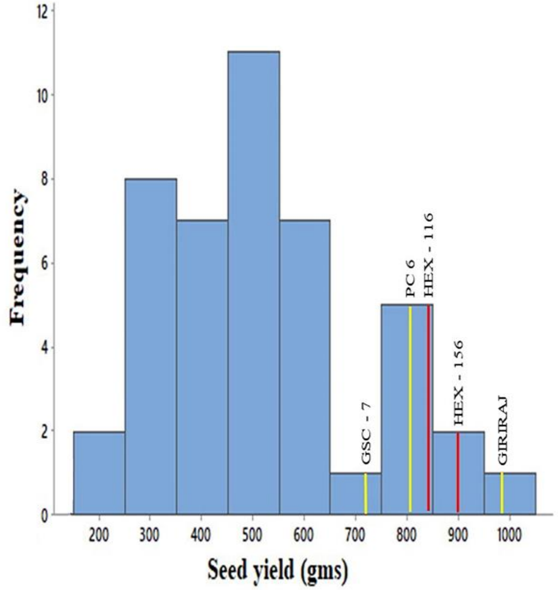
DAY NEUTRAL DEERMINATE *B.NAPUS*

BRASSICA ALLOHEXAPLOID









CONCLUSIONS

- The CAGR for yield improvement for rapeseed-mustard crops in India continues to hover around 1.8 percent, which needs to be doubled to meet the projected edible oil requirement of the country and also to account for the increasing use of vegetable oils for plant-based goods.

- Achieving such a level of genetic advance will require optimal deployment of available and introduced genetic diversity in improved cultivars genomic-assisted speed breeding and the use of gene editing tools.

- The development of across-species pangenomes and the use of genome editing techniques to eliminate linkage drag associated with introgressed variations will go a long way in developing disease and pest resistant cultivars.

- Gaps between potential and realized farm incomes are required to be bridged by incorporating environment resilience in high yielding varieties with enhanced product profiles (canola, extra-high-oleic canola, and enhanced protein quality).

- Improvement of all oilseed brassicas together can be optimized by fostering and financing broader international collaborations to encourage knowledge and germplasm exchanges.

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