



# जीनोमिक संसाधन संभाग भाकृअनुप - राष्ट्रीय पादप आनुवंशिक संसाधन ब्यूरो पूसा परिसर, नई दिल्ली - 110 012, भारत

## Division of Genomic Resources ICAR - National Bureau of Plant Genetic Resources

Pusa Campus, New Delhi -110 012, India



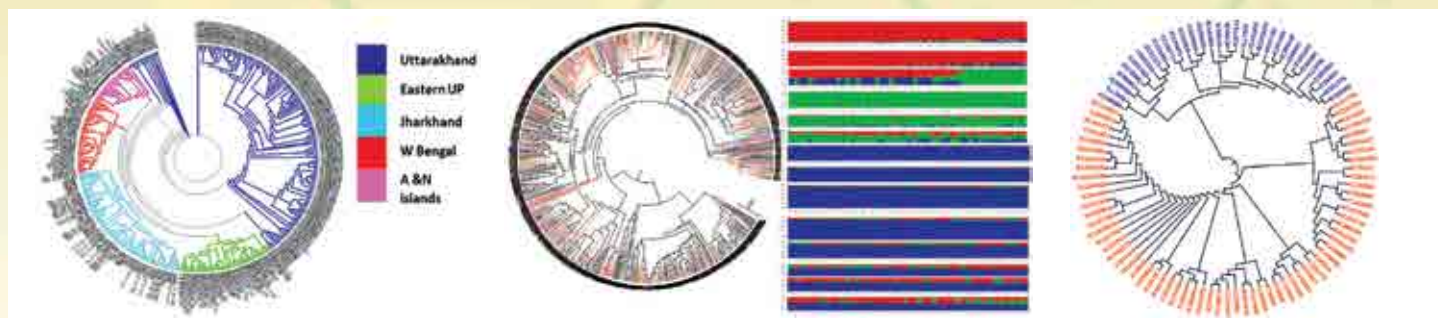
Division of Genomic Resources, ICAR-NBPGR, New Delhi was established in 2013 by reorganizing the erstwhile National Research Centre on DNA Fingerprinting with the following objectives:

- Development of genomic tools for enhanced utilization of crop germplasm
- Generation and conservation of genomic resources
- Molecular profiling of crop varieties
- Molecular diagnostics for GM detection

## GENETIC DIVERSITY ASSESSMENT

*Genetic diversity assessment provides opportunities to crop breeders to develop new and improved cultivars with desirable traits.*

Genetic diversity assessment was carried out in more than 20 agri-horticultural crops including cereals (rice, wheat, maize), millets (pearl millet, little millet, finger millet, kodo millet, foxtail millet, buckwheat), pulses (pigeon pea), oil seeds (*Brassica* spp., safflower, *Jatropha*), fibers (cotton, jute, flax) and horticultural crops (*Allium* spp., *Luffa*, bottle gourd, pomegranate, *Morinda* etc.)



Grouping based on HuSSR markers in rice based on geographical origin of selected 331 landraces confirming that landraces are preserved in their local niches.

Rice core of 701 accessions developed using 36 SNP markers in which 10% of total samples showed maximum diversity.

Neighbour-Joining phylogenetic tree of 96 lentil genotypes constructed using 1,27,204 SNPs discovered through whole-genome re-sequencing.

## DEVELOPMENT OF NEW DNA MARKER RESOURCES

*Genomics is providing new DNA marker resources, which are extremely useful for PGR characterization and utilization.*

Crop	Marker	Crop	Marker
Bitter gourd	70 genome wide SSRs	Little millet	1,305 putative EST-SSRs; 92 validated SSRs
Bottle gourd	801 EST-SSRs; 44,823 putative genome wide SSRs; 105 Validated SSRs; 207 Mapped SSRs	Moth bean	14,536 - SNPs
Brinjal	1,12,095 SNPs	Mung bean	38 -SSRs
Cucumber	12,450 SNPs	Okra	78,626 SSRs
Finger millet	3,883 putative EST-SSRs; 231 validated SSRs	Pigeon pea	7,34,810 genome wide SNPs; 328 genic SNPs
Giloe	5,412 EST-SSRs; 107 g-SSRs	Rice	95 miRNA –SSRs
Kalmegh	4,679 EST-SSRs; 61 g-SSRs	Ridge gourd	131 SSRs
Khus grass	36 SSRs	Sesame	70,000 – SSRs, SNPs
Kodo millet	1,437 putative EST-SSRs; 58 validated SSRs	Snake gourd	55 genome wide SSRs
Lathyrus	18,478 - SNPs	Sponge gourd	8,934 putative EST-SSRs; 191 Validated SSRs

SSR: Simple Sequence Repeat, SNP: Single Nucleotide Polymorphism, EST: Expressed Sequence Tags

### DNA Profiling and GM Detection Services

1. DNA profiling charges per sample: Rs. 5,000/- and Rs. 10,000/- (GST @ 18% additional) from public and private sector organizations, respectively.
2. GM testing charges per seed sample: Rs 5,000/- and Rs. 8000/- (GST @ 18% additional) from public and private sector organizations, respectively.



## DNA PROFILING

*DNA profiling leads to unequivocal identification of crop cultivars for protecting Plant Breeders' Rights.*

### Achievements:

- DNA profiling of 2,124 released varieties of over forty agricultural crops completed for their unique identification.
- DNA profiling service rendered to over six dozen public and private sector organizations as a requirement by Central Sub-Committee on Crop Standards, Notification and Release of Varieties.



DNA profile of maize genotypes with SSR marker BNLG 1325

### DNA Profiling Service Provided in 42 Crops

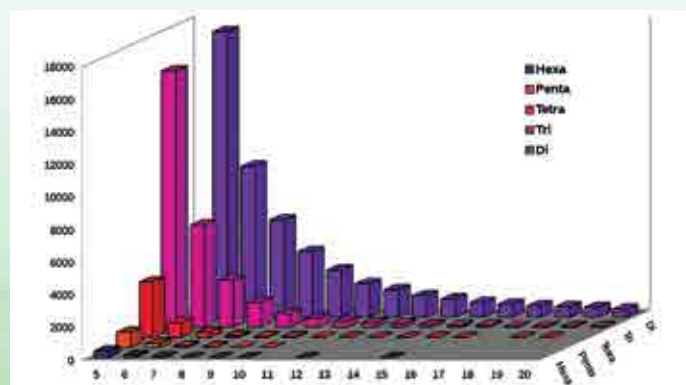
Amaranth	Cowpea	Jute	Melons	Pearl millet	Soybean
Barley	Cotton	Lab lab beans	Mothbean	Pigeonpea	Sorghum
Bitter gourd	Fennel	Lentil	Mustard	Rice	Sunflower
Black gram	Fenugreek	Linseed	Mungbean	Ricebean	Tomato
Brinjal	Finger millet	Little millet	Oats	Safflower	Toria
Chickpea	Foxtail millet	Maize	Okra	Saffron	Walnut
Chilli	French bean	Mango	Pea	Sesame	Wheat

## GENOME SEQUENCING & BIOINFORMATICS

*The DNA sequence information is valuable for identifying key genes controlling important agronomic traits and for identifying genetic variability in plants.*

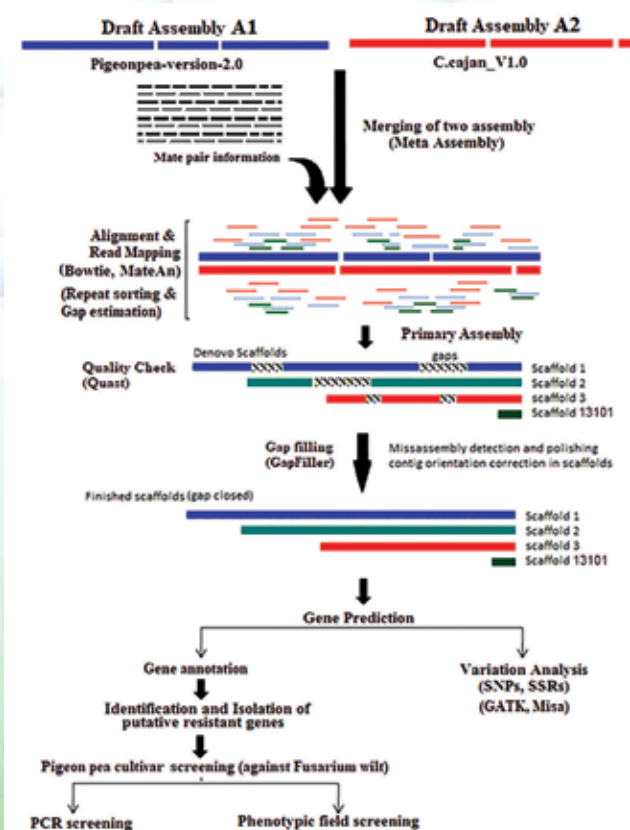
### Whole Genome Sequencing

Crop	Details
Black Pepper ( <i>Piper nigrum</i> ) landrace 'Thottumuriyan'	<ul style="list-style-type: none"> <li>◆ Annotated genes 12,725</li> <li>◆ Genome coverage 86%</li> </ul>
Ridge gourd ( <i>Luffa acutangula</i> ) Variety 'Pusa nutan'	<ul style="list-style-type: none"> <li>◆ Number of scaffolds 1,68,509</li> <li>◆ Genome coverage 37.3%</li> <li>◆ Gene number 21,803</li> </ul>
Sesame ( <i>Sesamum indicum</i> ) cultivar 'Swetha'	<ul style="list-style-type: none"> <li>◆ Number of scaffolds 76,029</li> <li>◆ Genome coverage 96.3%</li> <li>◆ Gene number 24,579</li> </ul>
<i>Solanum incanum</i>	◆ Re-sequencing of 15 landraces



Predicted SSRs (89,267) for *Luffa acutangula* variety 'Pusa Nutan' from whole genome sequences represented with SSR repeat frequency (X-axis: SSR repeat numbers, Y-axis: SSR frequency)

### Bioinformatics: In-house genome assembly pipeline

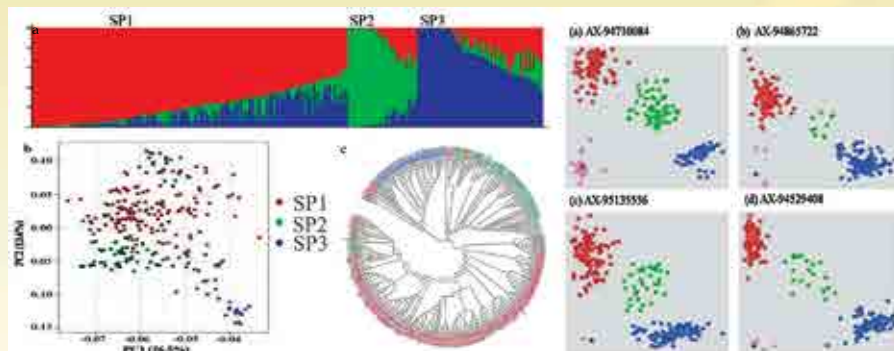


Improvement in pigeonpea genome coverage achieved from existing 72.0% in draft assemblies to 82.4% in finished genome by adopting new in-house developed assembly frame work pipeline and mate pair techniques.

## ENHANCED UTILIZATION OF GENOMIC TOOLS IN PGR RESEARCH “GENOPLASMICS”

*Utilization of genomic resources is possible by combining conventional breeding tools with genomic techniques and approaches or in other words by genomic-assisted plant breeding.*

**GWAS (Genome-Wide Association Studies) for resistance to spot blotch and development of KASP (Kompetitive Allele Specific PCR) markers in bread wheat**

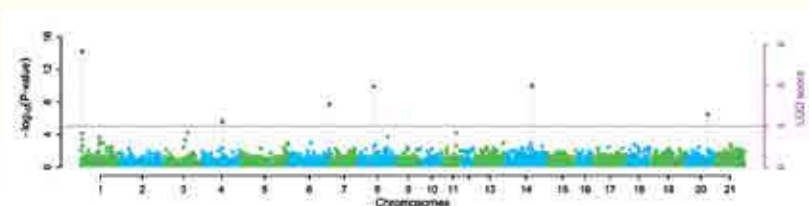


Three subpopulations in the selected association panel. (a) bar plot, (b) PCA and (c) Nj-tree.

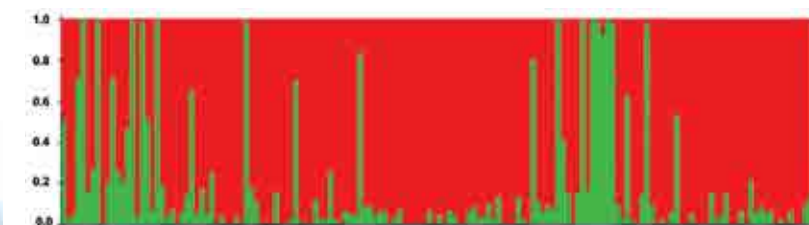
Genotypic evaluation of testing panel using KASP markers (a-d).

- A total of **seven significant MTAs/QTLs** were identified contributing **30.1 to 32.0%** to the total phenotypic variance.
- SNPs showing association with **spot blotch resistance** were identified for developing 10-15 KASP markers and were validated.

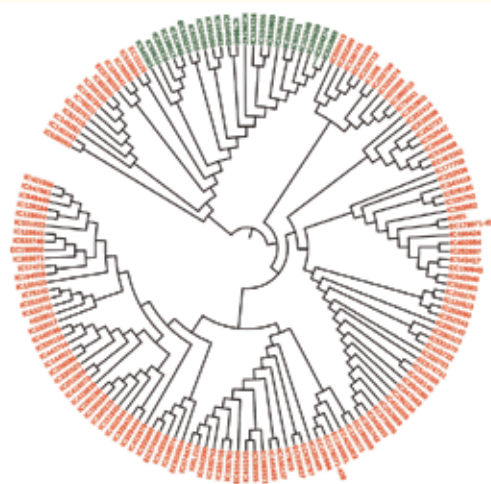
**GWAS identified twelve genomic regions in wheat for salinity tolerance traits**



Manhattan plot depicting significantly associated genomic regions for flag leaf  $K^+$  content

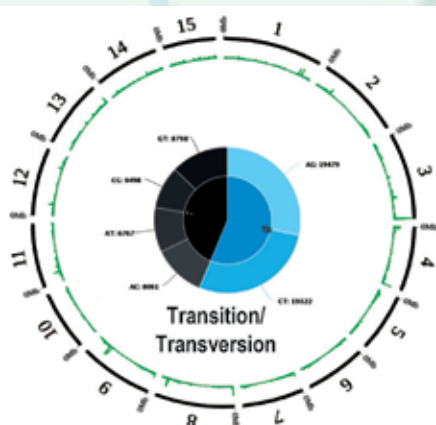


Population structure in wheat association panel

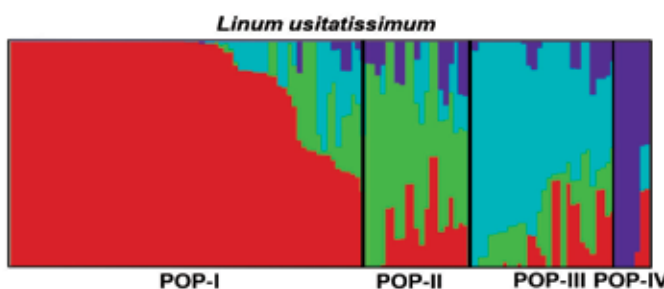


NJ tree of wheat association panel based on 15,495 SNP markers

**Genome wide SNPs in linseed identified using Genotyping By Sequencing (GBS) approach**



Distribution of SNPs (68,925) in all 15 chromosomes of linseed; Inner pie chart shows transitions and transversions



Population genetic structure of 131 linseed germplasm accessions using 68,925 SNPs



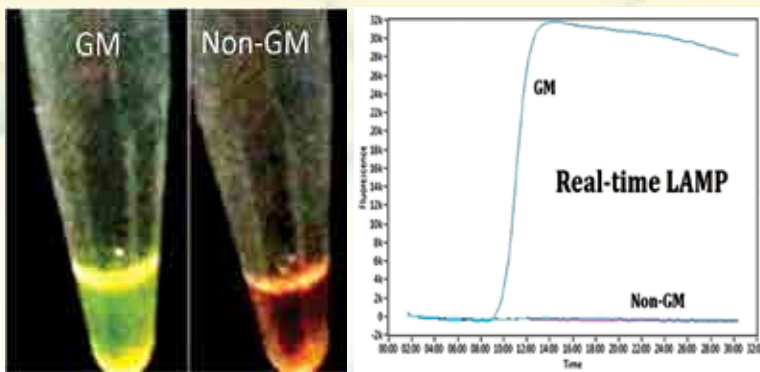
## GM DIAGNOSTICS

*GM Diagnostics play a key role to check for approved and unapproved GM events in the marketplace and supply chain for regulatory compliance.*

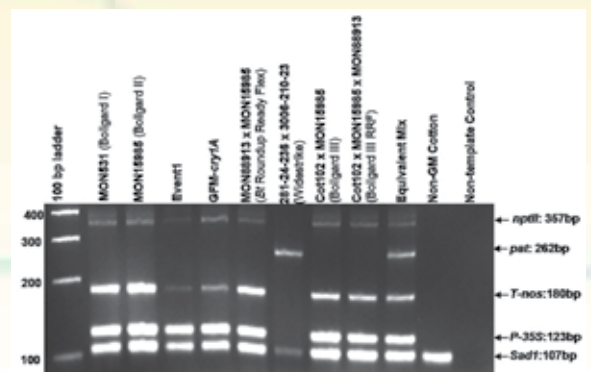
- PCR/ Real-time PCR, loop-mediated isothermal amplification (LAMP) based GM diagnostics conducted for more than 50 GM events of 15 crops including approved *Bt* cotton events.
- Molecular testing of more than 250 imports of 15 GM crops for specific targets and to ensure absence of embryogenesis deactivator gene was carried out.
- GMO testing service rendered to public/private sector organizations.

### GM free conservation in National Genebank

**D**NA-based diagnostics have been employed to monitor adventitious presence of transgenes in more than 700 accessions of cotton, brinjal, maize, okra and Indian mustard conserved in the National Genebank. None of the tested accessions showed adventitious presence of transgenes.



**Loop-mediated isothermal amplification (LAMP) based rapid/ on-site GM detection: >25 targets**



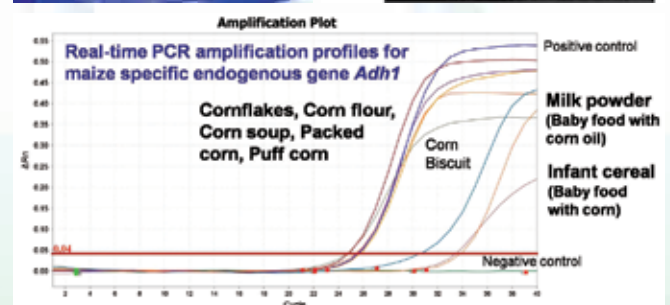
**Multiplex PCR: Screening of over 90% of globally approved GM cotton events**

### DNA extraction protocols for >20 food derivatives optimized and amplifiability confirmed

Crop	Food Derivatives
Mustard, Soybean, Canola, Cotton seed	Oil*
Maize	Popcorn, Cornflakes, Soup, Puff corn, Cornflour, Baby corn, Packed Corn, Biscuits, Infant cereal, Milk powder (corn oil)
Soybean	Milk, Nutrela (Soy chunks), Soy sticks, Tofu (Soy paneer)
Potato	Chips
Tomato	Soup, Sauce
Apple	Juice, Green apple

\*ISO/IEC 17025:2017 accreditation granted for GM detection in oil

### GM detection of food derivatives



**Confirming amplifiability using taxon-specific PCR/ Real-time PCR**

### GM Detection Research Facility (GDRF): Recognitions

- ❖ Accredited as per international standards ISO:IEC 17025:2017 by the National Accreditation Board for Testing and Calibration Laboratories (NABL), a Constituent Board of Quality Council of India.
- ❖ Designated as National Referral Laboratory to detect the presence or absence of LMOs and GMOs under sub-section (1) of Section 4 of the Seeds Act, 1966 in the Gazette of India Notification (DAC&FW, MoA&FW, Govt. of India) dated 15 November 2017.
- ❖ GDRF is coordinating the Network of GMO Testing Laboratories (NGTL) of India <http://gmolabs.nbprg.ernet.in:9090/>



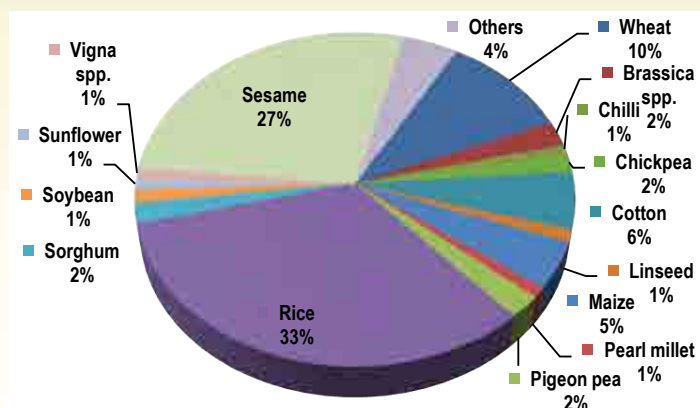
## CHECKING UNAUTHORIZED ENTRY OF GM CROPS

Adventitious presence of transgenes was monitored in 211 samples collected from Assam, Meghalaya, Mizoram, Tripura and West Bengal bordering Bangladesh. None of the samples showed adventitious presence of transgenes.

## CONSERVATION OF GENOMIC RESOURCES

*The conservation of genomic resources is indispensable for post-genomic research in PGR characterization, functional genomics, comparative genomics and molecular plant breeding.*

9,093 accessions from 45 cultivated and endangered plant species are being conserved at  $-80^{\circ}\text{C}$  and  $-196^{\circ}\text{C}$  in the National Genetic Resources Repository (NGRR) <http://www.nbpgr.ernet.in:8080/NPGR/Home.aspx>.

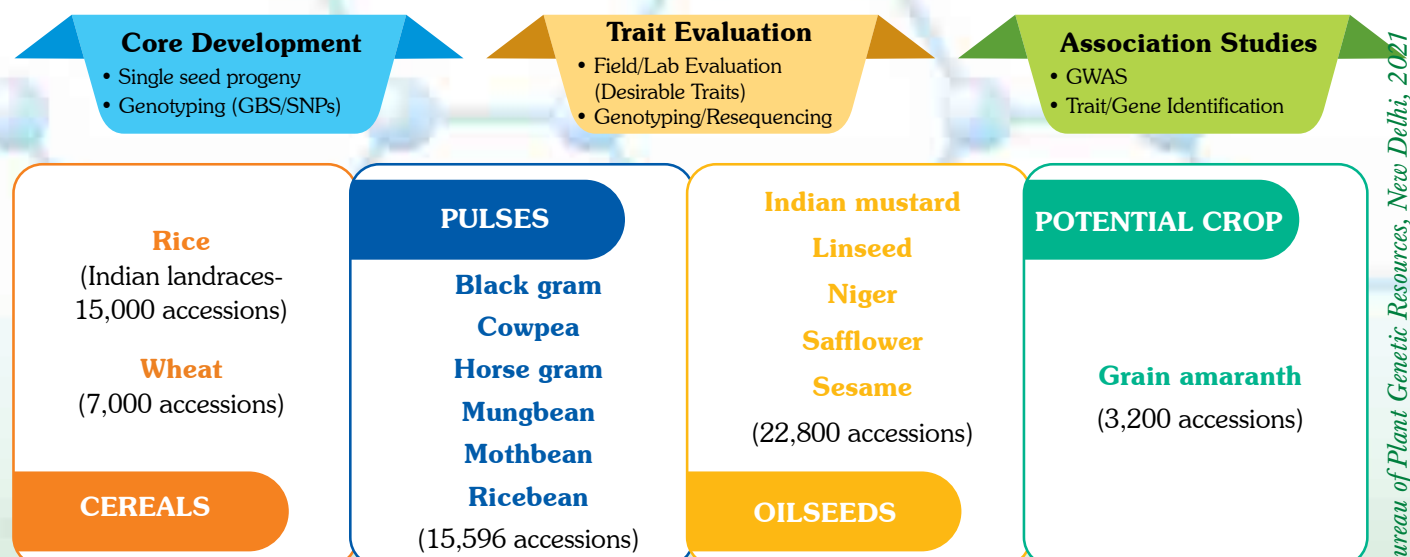


Crop-wise conservation status of genomic resources in NGRR



A snapshot of facilities at NGRR

## Comprehensive studies (funded by Department of Biotechnology, Govt. of India) on genomic insights undertaken in the crops conserved at National Genebank, India



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