



Leveraging Genetic Resources for Accelerated Genetic Improvements of Linseed using Comprehensive Genomics and Phenotyping Approaches

Under Mission Programme on "Minor Oilseeds of Indian Origin" Department of Biotechnology, Govt. of India

(2020-2023)
Major Achievements



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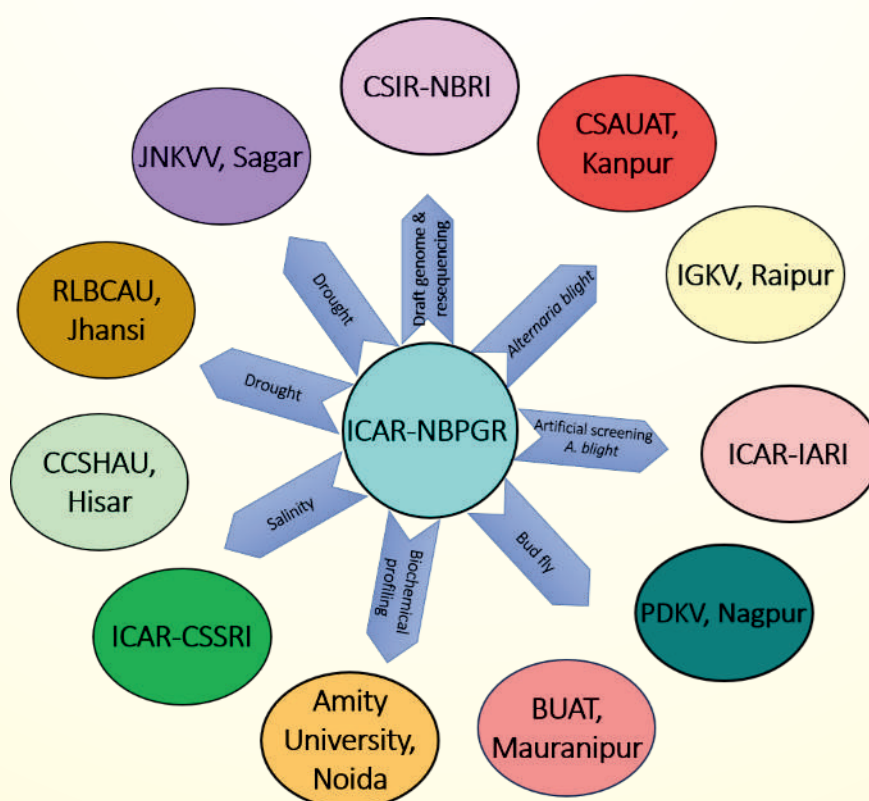


LINSEED RESEARCH NETWORK PROJECT

Linseed, an important oilseed of Indian origin, is amongst the earliest crops to have been domesticated. To address varied constraints in increasing linseed productivity, the whole collection of linseed germplasm (~2800 accessions) conserved at National Genebank (NGB), ICAR-NBPGR is being characterized and evaluated to identify superior germplasm accessions for important traits under the Linseed Network Project. The traits under consideration are key agromorphological traits, nutritional quality, (oil content, fatty acids, essential amino acids, proteins and lignans), resistance to biotic stress (*Alternaria* blight and linseed bud fly) and major abiotic stress (drought and salt stress). Further, to generate genomic resources, genome sequencing of one dominant variety of linseed (T-397) has been undertaken and a draft genome assembly has been prepared. All the gene bank accessions are being genotyped by re-sequencing to conduct Genome-Wide Association Studies (GWAS) and identify genomic regions associated with key agromorphological, nutritional traits as well as biotic and abiotic stress tolerance. The project will accelerate the genetic improvement of linseed and therefore a step towards higher productivity and production of an important indigenous oilseed for the benefit of farmers and the nation.

Coordinating Centre	ICAR-National Bureau of Plant Genetic Resources, New Delhi
Collaborating Institutes	12 (ICAR: 4, CSIR: 1, SAUs: 6, Private university:1)
Duration of project	5 years (2020-21 to 2024-25)
Date of sanction	29 February 2020
Total budget	Rs. 2255.8142 Lakhs

COLLABORATIVE RESEARCH INSTITUTES



MAJOR OBJECTIVES

- ❖ Multi-location phenotyping of entire linseed germplasm conserved at National Genebank for agro-morphological traits.
- ❖ Whole Genome Sequencing (WGS) of dominant variety (T-397) and re-sequencing of selected cultivated and wild germplasm.
- ❖ Development of composite core set (agro-morphological data and genome-wide SNP markers).
- ❖ Biochemical profiling of germplasm - Oil content, Protein content, Fatty Acid composition, essential amino acids, lignan (SDG) and development of NIR spectral prediction model for rapid and non-destructive estimation.
- ❖ Multi-location screening of entire germplasm at hot-spots and development of trait-specific reference sets for biotic (*Alternaria* blight, linseed bud fly) and abiotic stress (drought, salt)
- ❖ Genome-wide association mapping to identify genes/ markers associated with traits of economic importance.
- ❖ Augmentation of wild species and germplasm from diversity rich areas and genetic enhancement through wide hybridization.

TRAIT SPECIFIC EVALUATION

Trait	No. of accessions	Environment	Environment details (location-year)
Agro-morphological evaluation	2612 accessions	6	Delhi 2018-19, 2019-20, 2020-21 and 2021-22 Akola 2020-21 and 2021-22
Biochemical profiling	2640 (Fatty acid) 2640 (SDG lignan) 2640 (Amino acid) 2612 (Oil content) 2663 (Protein content) 2612 (Proximate analysis) 30 (Polyphenol content)	-	Biochemical profiling done from seed multiplied through single plant progenies. Validation of superior accessions from seed harvested from multilocation-year trials is under progress.
<i>Alternaria</i> blight resistance	2612	4	Kanpur 2020-21 and 2021-22 Raipur 2020-21 and 2021-22
	244 (Artificial epiphytotic conditions)	2	IARI 2022-23 and 2023-24
Linseed bud fly resistance	2612	4	Nagpur 2020-21 and 2021-22 Mauranipur 2020-21 and 2021-22
Drought stress tolerance	2612	6	Delhi 2020-21 and 2021-22 Jhansi 2020-21 and 2021-22 Sagar 2020-21 and 2021-22
Salinity tolerance	2612	4	Karnal 2020-21 and 2021-22 Hisar 2020-21 and 2021-22
Alkalinity tolerance	2612	4	Karnal 2020-21 and 2021-22 Kanpur 2020-21 and 2021-22

BIOTIC STRESS

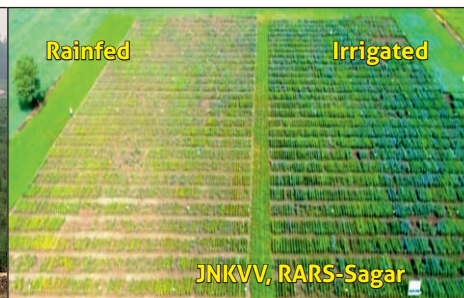


Field screening and validation of resistance under artificial epiphytotic condition of resistance to *Alternaria* blight



Field screening of 2612 accessions for resistance to bud fly and rearing of bud fly

ABIOTIC STRESS



Evaluation of 2612 linseed accession for drought tolerance during 2020-21 and 2021-22

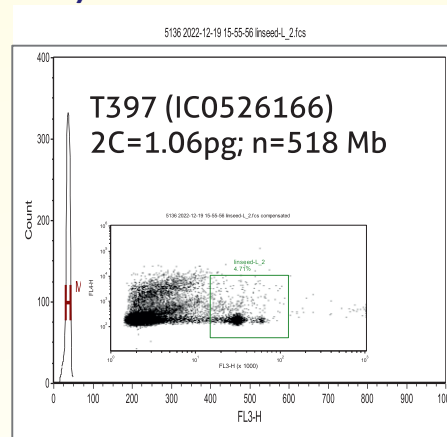


Aerial view of entire linseed germplasm being evaluated under control, alkaline and saline conditions at ICAR-CSSRI, Karnal

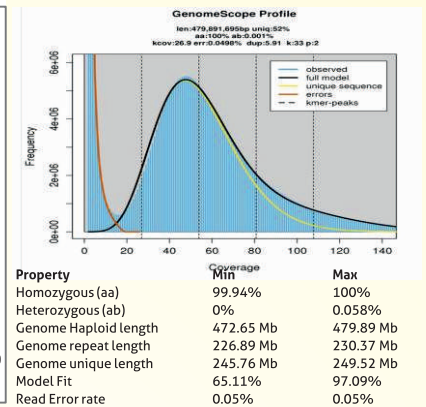
DEVELOPMENT OF REFERENCE GENOME

Statistics of hybrid genome assembly (T-397)

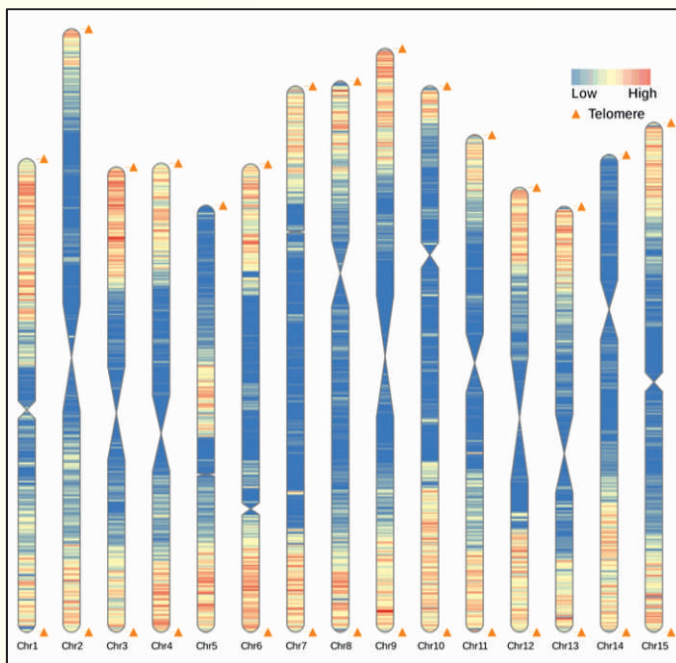
Genomic feature	T397
Total Assembly size	494.85 Mb
Number of scaffolds	15
Largest scaffold	39.86 Mb
Average scaffold length	32.99 Mb
scaffolds N50	32.86Mb
L50	7
L90	14
Number of gaps	5
N counts	4726
GC content	37.20%
N' per 100 kbp	0.96



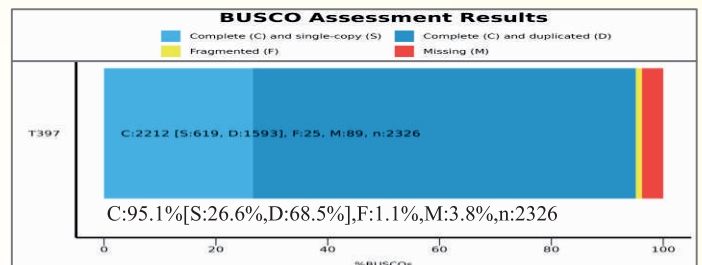
Flow cytometry estimated genome size of T-397 (~518 Mb)



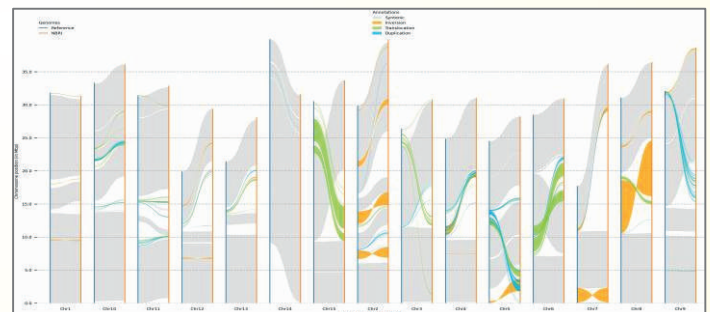
K-mer based estimated genome size of T-397 (~480 Mb)



Linseed genome karyotype showing centromere (15), telomere (29) and gene density across the 15 chromosomes



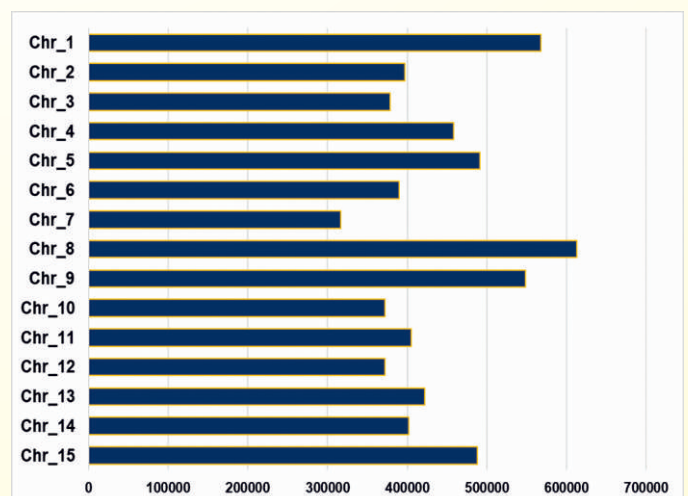
Genome completeness of T397 assessed using BUSCO genes (Eudicot db)



Syntenic and structural variation between T397 genome and YY5 (Sa et al. 2021) genome

GENOTYPING

- ❖ Resequencing for 2000 accessions (~5X depth) has been completed.
- ❖ Variant calling showed 38,91,279 total SNPs and 2,43,278 SNPs with Minor Allele Frequency >0.05.
- ❖ ML-GWAS led to the identification of 84 unique significant QTNs comprising 30 stable QTNs for Thousand Seed Weight.
- ❖ Total 23 candidate genes have been identified for TSW, which included shaggy-related protein kinase/BIN2, ANTIAUXIN29 RESISTANT 3, RING-type E3 ubiquitin transferase E4, auxin response factors, WRKY transcription factor etc.



Chromosome wise distribution of SNPs

TRAIT SPECIFIC GENOTYPES

Earliness: 24 genotypes for NW plain zone (<120 days to maturity) and 126 genotypes for Deccan plateau (<100 days to maturity).

Trait enriched germplasm for nutritional/nutraceutical/industrial utility:

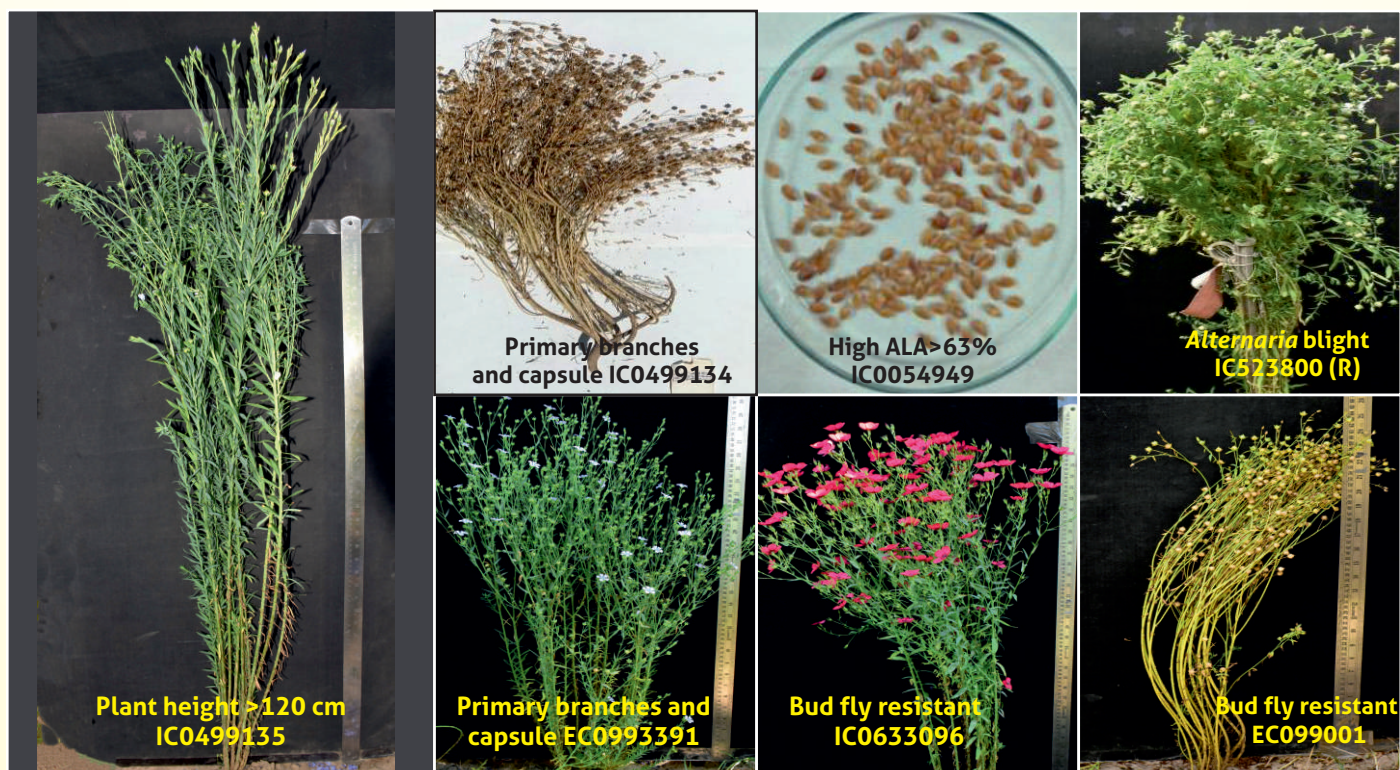
- Protein content ($\geq 23\%$)- 30 accessions
- Oil content ($\geq 45\%$)- 65 accessions
- High ALA (omega 3 FA) content $\geq 60\%$ - 18 accessions
- Low ALA (<30%)- 2 accessions (IC0096572; IC0498905)
- High Threonine (>20%)- 18 accessions
- High Histidine (>15%)- 13 accessions
- High SDG (>2500000 ug/g)- 45 accessions

Alternaria blight resistant accessions identified and validated- Six accessions namely- IC0385354, IC0499104, IC0523800, IC0591124, EC0718850 and IC0498580, were validated for resistance against two isolates of *A. lini* (Delhi and Kanpur) under artificial epiphytotic conditions.

Bud fly resistant accessions identified and validated- IC0499071, IC0498795, IC0498763, EC099001, IC618739, EC993391, IC633096 and EC099001 validated across multiple location-year trials.

High yielding genotypes under drought stressed and non-stressed conditions- Stress Tolerance Index (STI>0.7): EC0041466, EC0455084, EC0541226, IC0096530, IC0096540, IC0096601, IC0356276, IC0385343, IC0385397, IC0498992, IC0499176, IC0525941, IC0526133, IC0606067, IC0629218, IC0629220.

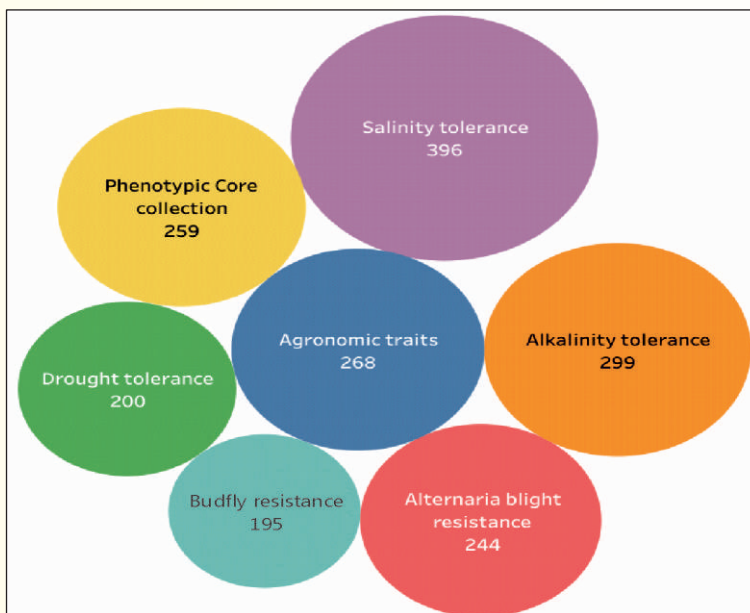
For salt tolerance 170 accessions tolerant (STI > 0.65) under saline (8-10 dS/m) conditions and 229 accessions tolerant (STI > 0.65) under sodic (pH 9.5 \pm 0.2) conditions were identified.



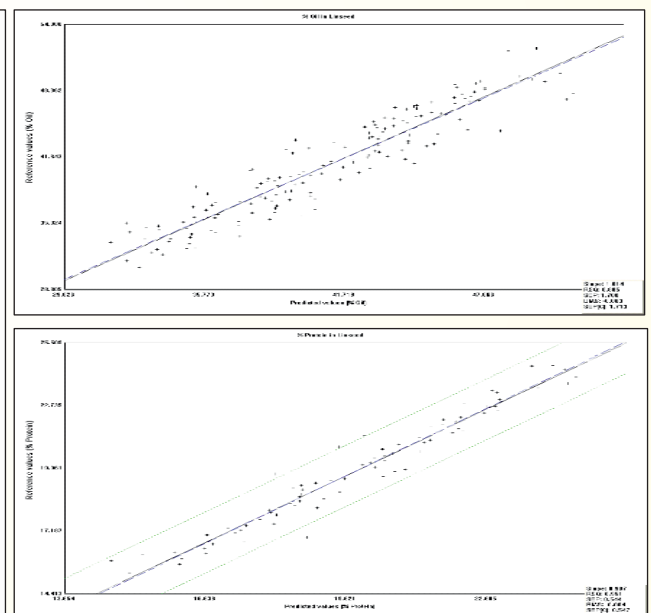
Trait specific genotypes

MAJOR ACCOMPLISHMENTS

- Core collection (259 accessions) based on 36 agro-morphological and nutritional traits.
- Trait-specific reference sets (total 268 accessions) for agronomic traits (Flowering & Maturity- 75; Bold Seeds & Capsules- 59; Plant Height & Technical Height- 44; TSW & Seed Yield- 29; Capsules/Plant- 24; Seeds/Capsule- 10; Early Plant Vigour- 27).
- Reference set (200 accessions) for drought tolerance .
- Reference set (396 accessions) for salinity tolerance (8-10 dS/m).
- Reference set (299 accessions) for alkalinity tolerance (pH 9.5±0.2).
- Reference set (244 accessions) for *Alternaria* blight resistance.
- Reference set (195 accessions) for linseed bud fly resistance.
- A quick analysis method for protein quantification in oilseed crops.
- Draft genome assembly of popular linseed cultivar T-397.
- IC0384578 registered as genetic stock (INGR23054) for high number of capsules.
- NIRS prediction model for oil and protein content for high throughput and non-destructive estimation of biochemical constituents.
- Protocol for embryo rescue in abortive crosses and successful plantlet regeneration.



Trait-specific Reference Sets



NIR prediction for oil and protein content

INFRASTRUCTURE



Glass house facility



Stress physiology and Molecular biology laboratory

PUBLICATIONS

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Acknowledgements: We are thankful to DBT, Gol for funding Support and ICAR-NBPGR for facilities.

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