

Progress Report

Utilizing chickpea genome sequence for crop improvement

Submitted to
Department of Agriculture and Cooperation (DAC)
Ministry of Agriculture, Government of India

in collaboration with
International Center for Agricultural Research in the Dry Areas (ICARDA)
- New Delhi
Indian Institute of Pulses Research (IIPR) - Kanpur
Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya (RVSKVV),
RAK College of Agriculture (RAKCA) - Sehore
Rajasthan Agricultural Research Institute – Durgapura
Junagadh Agricultural University - Junagadh



**International Crops Research Institute
for the Semi-Arid Tropics**

This work is being
undertaken
as part of the



RESEARCH
PROGRAM ON
Grain Legumes

Project Profile

- Project title:** Utilizing chickpea genome sequence for crop improvement
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Institutes and Scientists Involved:

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Project duration: Three years

Total cost of the project: INR 1264.85 lakhs

Project Goals

A consortium of leading chickpea breeders and genomics scientists from different institutes of India was constituted for using chickpea genome for accelerating chickpea breeding to identifying the molecular basis of key agronomic traits.

Key objectives

1. Evaluation of existing superior lines, based on available data, in multi-location environments
2. Molecular breeding for higher grain yield by integrating genomic selection approach in chickpea breeding
3. Molecular mapping for important target traits for chickpea using nested association mapping (NAM) approach
4. Mapping of targeted traits and harnessing the germplasm diversity using genome-wide association study (GWAS) approach
5. Development of breeders' friendly database
6. Empowering Indian chickpea breeders and scientists in modern genome analysis for chickpea improvement

Expected outcomes

Project has been successfully initiated with following possible outcome:

- (i) Release of molecular breeding products for drought tolerance
- (ii) Genomic selection (GS), a new molecular breeding approach integrated in chickpea breeding programmes in India
- (iii) Molecular markers for target traits for developing next generation of superior varieties
- (iv) Databases with genotyping and phenotyping information for chickpea breeding community
- (v) Next generation of scientists with community of practice of molecular breeding in chickpea in India

Project Meetings

Planning meeting

In order to utilize the chickpea season, after receiving the administrative approval of from Department of Agriculture and Cooperation (DAC), Ministry of Agriculture, ICRISAT organized a two day planning meeting at ICRISAT on Sept 16-17, 2014 to finalize the work plan for each centre so that they can undertake the respective activities. Scientists/representative from all the partner Institutes were present and participated in the objective-wise discussions and contributed for finalization of work-plan and project material (Fig 1).

Annual review and project planning meeting

With an objective to review the project and progress and plan the activity for year 2015-16, annual review and planning meeting was organized at IIPR, Kanpur on Apr 11, 2015. Dr. D.P Malik (Additional Commissioner (Crops), DAC), also attended the review and planning meeting and appreciated all the partners for their achievement (Fig 2). Scientist from all the partner institutes attended the meeting and presented the project progress at their respective centre (Fig 3). In addition to review and planning meeting, field visit to see the project experiments for all the partners was organized (Fig 4).

Technical Report (2014-15)

Executive Summary

Chickpea is the second most important food legume that highly nutritious and protein rich source and contribute to income generation and improved livelihood of small-holder farmers in India. India is the largest producer of chickpea covering about 65% of global chickpea production. India being the largest consumer of chickpea imports about 1.5 million tons of chickpea annually. Recently, draft genome of chickpea became available which can help in improving grain yield, quality, greater drought tolerance, disease resistance and enhanced genetic diversity. The genome sequence helps with wide range of studies, from the important goal of accelerated breeding to identifying the molecular basis of key agronomic traits, in addition to understanding the basic legume biology. Chickpea genome can also help in developing superior varieties tolerant to abiotic and biotic stresses as well as early maturing varieties.

After the completion of draft genome sequence this project is leading efforts to utilize genome sequence for developing superior chickpea lines using range of approaches including marker assisted backcrossing (MABC), genomic selection (GS), nested association mapping (NAM), linkage mapping and genome-wide association study (GWAS). Efforts are also underway to develop a cloud based breeder friendly database, so that genotyping and phenotyping data, marker-trait association information can be stored in structured query language (SQL) database and make available to breeders to select the superior lines or markers/genes in their future breeding programs. Under this project RILs populations are being genotyped using high-throughput genotyping using 50K SNP array. Genotyping data along with phenotyping data will be used for defining

the statistical genomic selection models for deploying GS in chickpea breeding. For NAM population, 14 different crosses have been made having ICC 4958 as common female parent. Around 3000 NAM lines will be genotyped using high-throughput genotyping. Genotyping data along with phenotyping data on these NAM lines will be used for identification of markers associated with trait of interest. Similarly for GWAS, 3000 lines from composite collection are being re-sequenced at 10X coverage using whole genome re-sequencing (WGRS) approach. Out of these 3000, around 1000 lines have already been sequenced generating 7-10 Gb sequence data for each line. In parallel, all these 3000 lines were phenotyped at all the partner location in 2014-15 chickpea crop season. Multi-location phenotyping data on these 3000 lines for two years will be used along with re-sequence data for undertaking GWAS. Each centre is actively involved and contributing to make the project successful.

Objective-wise progress report

Objective 1: Evaluation of existing superior lines, based on available data, in multi-location environments

Activities:

- 1.1 Selection of 100-200 lines of multi-location evaluation*
- 1.2 Multi-location evaluation of lines for yield and yield related traits*
- 1.3 Selection of better performing lines for each region*

Superior chickpea varieties developed by all the partner institutes using modern and conventional breeding approaches as part of several projects were selected. Based on the discussions during project planning meeting a set of 100 such superior chickpea varieties was constituted by contribution from all the centres as follows:

ICARDA	8 lines
IIPR	10 lines
JAU	8 lines
RAKCA	8 lines
RARI	20 lines
ICRISAT	40 lines
Local/regional checks	6 lines
Total	100 lines

In total, 94 superior chickpea varieties were contributed from 6 partner centres. In addition, 6 local/regional checks were included to see the performance of selected elite lines with respect to the check. Regional check included JG 16 (Sehore), GG-4 (WB), GG-1 (JAU), RSG-888 (Jaipur), JG 11 (ICRISAT).

Based on the discussions in the meeting, each centre sent one kg seed for each lines to ICRISAT for constitution of set of 100 lines. After the constitution of whole panel, ICRISAT shared the seed material (100 lines) with all the centres for evaluation. Panel of 100 lines including local/regional checks was evaluated in Alpha-lattice design at each location in 2014-15 chickpea cop season. Selected six locations are ICARDA (Kalyani, West Bengal), IIPR (Kanpur), JAU (Junagarh), RAKCA (Sehore, MP), RARI (Jaipur, Rajasthan) and ICRISAT (Patancheru, Telangana). For the evaluation, 4 row of 4 meter length in three replications were used. Based on the discussions in the planning meeting traits to be

measured (days to 50% flowering, days to maturity, plant height (canopy height), grain yield/plot, 100 seed weight, no of plants harvested) were decided (Fig 5).

Data recording at each centre is almost completed and compilation of data is underway. Based on the discussion during annual review and planning meeting, all the partners will share the data with ICRISAT. After receiving data from all the centres, ICRISAT will organize 1-2 day workshop, where Scientists from all the centres will be invited and data will be analysed all together.

Objective 2: Molecular breeding for higher grain yield by integrating genomic selection approach in chickpea breeding

Activities: 2.1 Genotyping of populations for genomic selection using GBS approach
 2.2 Multi-location phenotyping for phonological, yield and yield related traits for two seasons
 2.3 Selection and crossing of superior lines based on GEBVs

Under this objective, ICRISAT is leading efforts for deploying genomic selection (GS) while IIPR is undertaking the linkage mapping approach for mapping the target trait. ICRISAT has developed two bi-parental populations (ICC 283 × ICC 8261 and ICC 4958 × ICC 1882) with about 250 RILs in each population. GS helps in predicting the individual's breeding value by selecting of an individual prior to phenotyping based on genome-wide marker data. ICRISAT as part of several other initiative has developed the 50K SNP array for high density genotyping. Genomic DNA from all the RILs of both the population was isolated and is being used for high-throughput genotyping using 50K SNP array. In parallel, existing phenotyping data for yield related traits on these RILs is being compiled. After the completion of genotyping, genome-wide genotyping data along with multi-location phenotyping data will be used for defining the GS models for yield and yield related traits. Superior RILs based on higher genomic estimated breeding values (GEBVs) will be selected.

Similarly, IIPR had developed two mapping population namely WR 315 × JG 62 (Fusarium Wilt) and K 850 × IPC 04-52 (100 seed weight). Both these population will be genotyped using high-density 50K SNP array. In parallel, these populations are being phenotyped for target traits. Genotyping data along with phenotyping data will be used for mapping of target traits using linkage mapping approach.

Objective 3: Molecular mapping for important target traits for chickpea using nested association mapping (NAM) and linkage mapping approach

Activities: 3.1 Develop 12 bi-parental populations using ICC 4958 as a common parent and other bi-parental population segregating for wilt and seed size
 3.2 Genotyping of NAM and bi-parental populations using GBS approach
 3.3 Multi-location phenotyping of NAM populations
 3.4 Establish marker/haplotype/gene- trait association

The NAM population is developed having one common parent, with the other parental genotype contrasting for traits of interest to the common parent genotype that lead to generation of new breeding material with enhanced diversity. Based on the discussions

in the planning meeting, each partner centre undertook the crossing of two elite chickpea line with ICC 4958 as common female parent (Table 1). As discussed in planning meeting, ICRISAT shared seeds for ICC 4958 with each centre so that all centre use same genotype for crossing. All the centres have undertaken the crossing as planned. Based on discussions in planning meeting, project will be using the off season sowing facility at Dharwad. In this regard, seeds from F₁ have been harvested and shared with IIPR for sowing at Dharwad to make use of off season. DNA from all the F₁s will be isolated from Dharwad to check the hybridity of F₁s.

In addition, further to add the value of project, MABC is being used for introgressing the drought responsive “QTL-hotspot” in to the elite chickpea lines from ICC 4958. ICRISAT has already introgressed this “QTL-hotspot” in JG 11, elite chickpea cultivar. Introgression lines has shown improved performance under rainfed as well as irrigated environment as compared to recurrent parent. Inspiring by the success of this, project plans to introgress the “QTL-hotspot” in all the elite varieties that are being used for developing NAM population.

Table 1: List of NAM crosses being undertaken at each centre

S. No.	Centre Name	NAM crosses
1.	IIPR, Kanpur	ICC 4958 × T39-1
		ICC 4958 × DCP92-3
2.	RAKCA, Sehore	ICC 4958 × RVG 202
		ICC 4958 × JG 6
3.	JAU, Junagarh	ICC 4958 × GG 2
		ICC 4958 × GJG 3
4.	RARI, Durgapura	ICC 4958 × RSG-965
		ICC 4958 × CSJ-160
5.	ICRISAT, Patancheru	ICC 4958 × ICCV 10
		ICC 4958 × JG 11
		ICC 4958 × JG 14
		ICC 4958 × JAKI 9218

*Although IARI is not a part of consortium, but they are also developing two crosses (ICC4958 × PUSA 547 and ICC 4958 × PUSA 372) for NAM population

Objective 4: Mapping of targeted traits and harnessing the germplasm diversity using genome-wide association study (GWAS) approach

- Activities:*
- 4.1 Genotyping of the global composite collection using WGRS/GBS approach*
 - 4.2 Multi-location phenotyping of global composite collection at least for two years*
 - 4.3 Establish marker/haplotype/gene- trait association*

With an objective to understand the genetic diversity in available germplasm, re-sequencing of global composite collection of 3,000 accessions has been initiated with support from this project. All the 3000 lines are being re-sequenced at min 10X coverage using whole genome re-sequencing (WGRS) approach on illumina Hiseq platform at ICRISAT. Total genomic DNA from all 3000 lines was extracted from 10-12 leaves of two week old plants following a modified CTAB protocol. Paired-end sequencing libraries with insert sizes of ~200 bp or 500 bp were constructed for accessions according to the manufacturer's instructions (Illumina) and are being used for sequencing. So far around 1000 lines have already been sequenced with 7-10 Gb sequence data generated for each line.

In parallel, all these 3,000 lines were evaluated at IIPR, RARI, RAKCA, JAU, ICARDA and ICRISAT in non-replicated augmented design (Fig 6 & 7). Seeds for all these lines were shared with all the partners. Based on the discussions in the planning meeting following traits were decided:

- ❖ Days to 50% flowering (Plot basis)
- ❖ Days to maturity (Plot basis)
- ❖ 100 seed weight (Plot basis)
- ❖ Yield of lines (No of plants at maturity and Seed yield/plot)
- ❖ Data on selected 5 lines (Random selection)
 - Plant height
 - Primary branches
 - No of pods/plant
 - Yield/plant

After completion of re-sequencing of all 3000 lines, WGRS data will be aligned against reference genome for variant calling and other structural variation analysis. Genotyping data along with phenotyping datasets will help identification of superior haplotypes for targeted traits that can be deployed in the chickpea breeding programme.

Objective 5: Development of breeders' friendly database

- Activities:*
- 5.1 Curation and assembling of genotyping, phenotyping and trait-association data*
 - 5.2 Develop breeder friendly database for trait, marker, germplasm lines*

Huge amount of genotyping and phenotyping data generated under this project is a significant outcome of this project that can provide significant contribution to the chickpea pre-breeding, and chickpea breeding communities. ICRISAT is working to develop a cloud based database so that all the project data can be uploaded and accessed from anywhere with all the project partners. In addition, ICRISAT is also working with

BMGF initiative called “Global Open-source Breeding and Informatics Initiative (GOBII)” to make all the data available freely.

Objective 6: Empowering Indian chickpea breeders and scientists in modern genome analysis for chickpea improvement

Activities: 6.1 Empowering of scientists in genomics and molecular breeding techniques
 6.2 Participation of breeders and scientists in workshops

Developing next generation of Scientists is very important for strengthening the national crop improvement programmes. Under this project chickpea scientists will be trained on advanced molecular breeding approaches and utilization of the decision supporting tools by conducting a range of “Annual and Product Dissemination Workshops” with consortium partners and chickpea research community. In this regard, ICRISAT has organized a three day international conference on 5th International Conference on Next Generation Genomics and Integrated Breeding for Crop Improvement (NGGIBCI-V) during February 18-20, 2015 which was attended by >350 registered participants from 30 different countries. Chickpea scientists from all the partners were invited to attend the conference so that they get chance to interact with the experts of respective field and make them aware about the recent updates in the field of next generation genomics. Scientists from partner institutes including ICARDA, IIPR and ICRISAT attended the conference and got a chance to interact with international community to understand the recent development in the field of next generation genomics.



Fig 1: Participants of the DAC project planning meeting held on September 16-17, 2014



Fig 2: Dr. D.P Malik (DAC), Dr. N.P. Singh (IIPR), Dr. Rajeev Varshney (ICRISAT), and Dr. P.M. Gaur (ICRISAT) during Annual Review and Planning Meeting held on Apr 11, 2015 at IIPR, Kanpur



Fig 3: Scientists from all the partner centres along with IIPR scientists attending the annual review and planning meeting held at IIPR Kanpur, on April 11, 2015



Fig 4: Scientists visiting chickpea field at IIPR, Kanpur



Fig 5: Field trial of selected 100 superior chickpea lines for yield and yield related traits sown in three replication. Each replication having 4 row/line

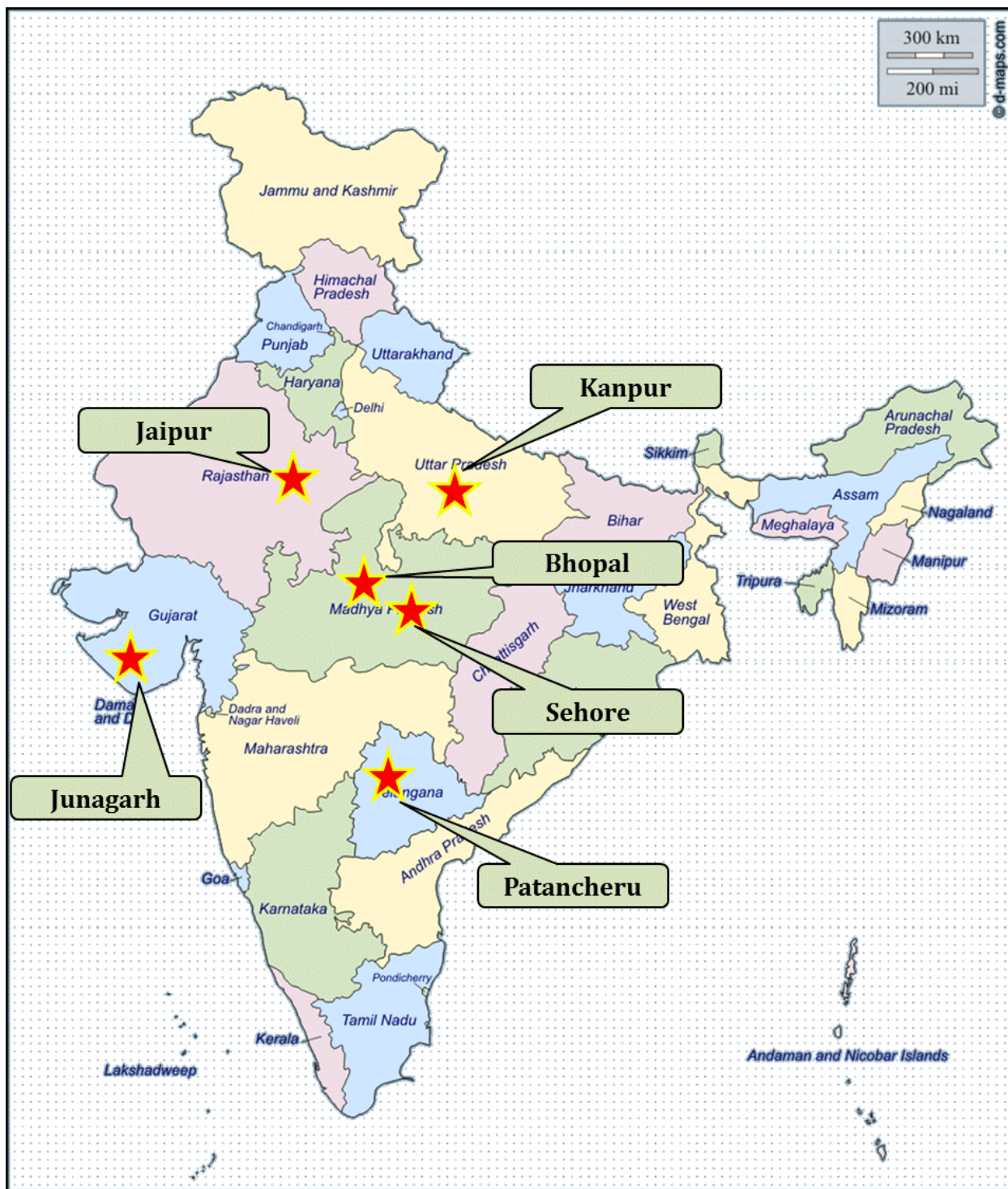


Fig 6: Geographical representation of chickpea field evaluation locations across India



Fig 7: Field evaluation of 3000 lines from chickpea composite collection for yield and yield related traits

Photos from field visits on different partner centres



ICRISAT Scientists visiting RARI, Durgapura



ICRISAT Scientists visiting RAKCA, Sehore