



NEXT-GENERATION BREEDING FOR CLIMATE-RESILIENT CHICKPEA (*CICER ARIETINUM* L.)

Ankit Kumar, Vivek Singh, Jogendra Singh and
Vijayata Singh*

ICAR-Central Soil Salinity Research Institute, Karnal 132001, India



INTRODUCTION

The multi-omics revolution has fundamentally restructured our understanding of how chickpea mounts its molecular defence against environmental adversity. Where earlier research relied on single-gene candidate approaches, contemporary transcriptomics, proteomics, and metabolomics now deliver system-wide snapshots of the stressed plant simultaneously revealing regulatory hubs, protective metabolites and protein-level adaptations that no single-gene study could uncover (Figure 1).

TRANSCRIPTOMICS AND RNA-SEQ

RNA sequencing (RNA-seq) has emerged as the workhorse of stress-response characterisation in chickpea. A landmark study on the drought-tolerant genotype ICC 4958 identified 15,947 differentially expressed genes (DEGs), including the transcription factor superfamilies

MYB, NAC, WRKY, bHLH, and bZIP master regulators that control hundreds of downstream stress-response genes. For salinity, analysis of the tolerant J11 genotype revealed 21,698 DEGs, subsequently assigned to 64 functional categories with emphasis on potassium transporters (HAK/KUP), aquaporins (MIP family) and NADH dehydrogenase subunits.

Perhaps the most important finding from transcriptomics has been the confirmation that stress-response gene networks are highly context-dependent. 4,954 genes were uniquely regulated in drought-resistant genotypes while 5,545 were exclusive to salt-tolerant lines — suggesting that molecular tolerance strategies are stress-specific rather than generic, a finding with immediate implications for targeted marker development.

PROTEOMICS

Proteomic analyses complement transcriptomics by capturing post-translational

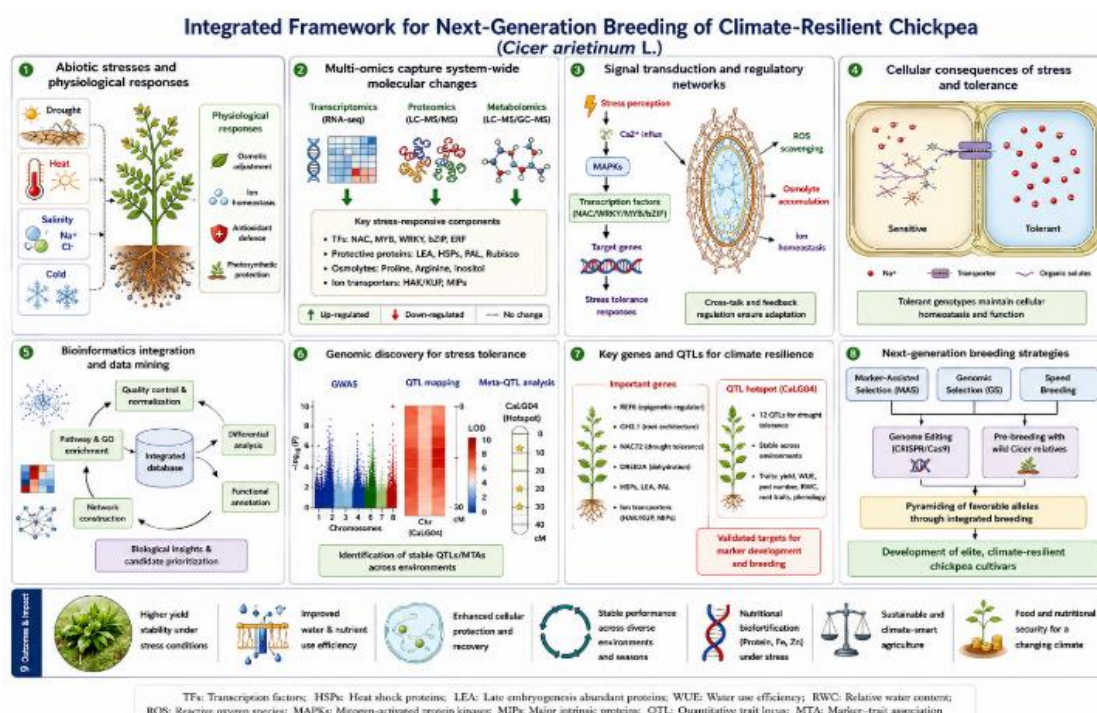


Figure 1. Integrated framework for next-generation breeding of climate-resilient chickpea (*Cicer arietinum* L.) using multi-omics, bioinformatics and advanced breeding technologies.

modifications that mRNA profiling misses. Identified 134 dehydration-responsive proteins in the drought-tolerant JG-62 genotype, partitioned across metabolism, signal transduction, cellular defence and cell wall remodelling pathways. In heat stress research, characterised 482 heat-responsive proteins in genotype JG14, including phenylalanine ammonia-lyase (PAL 2), late embryogenesis abundant (LEA) proteins, and RuBisCO subunits all linked to maintaining photosynthetic integrity under thermal load.

METABOLOMICS

The metabolome provides the final layer of molecular resolution and significant accumulation of L-arginine, L-proline, L-isoleucine, L-histidine, and tryptophan in drought-tolerant genotypes amino acids functioning as osmoprotectants maintaining cell turgor under water deficit. Desi chickpea genotypes accumulate significantly higher concentrations of inositol and threonic acid under rainfed conditions, implicating inositol phosphate biosynthesis as a core drought resistance mechanism.

ROLE OF BIOINFORMATICS

The sheer volume of data generated by modern omics platforms renders traditional statistical approaches inadequate. Bioinformatics has therefore become the indispensable bridge between molecular data generation and actionable breeding intelligence, providing the computational frameworks needed to extract meaning from millions of SNP markers and thousands of differentially expressed genes.

GENOME-WIDE ASSOCIATION STUDIES (GWAS)

GWAS has emerged as the most powerful tool for dissecting the genetic architecture of complex, quantitative traits such as drought and heat tolerance. A landmark study on 300 chickpea accessions characterised 312 significant marker-trait associations (MTAs) for drought and heat stress traits. 429 global accessions and 3.65 million SNPs, identifying 262 MTAs including associations with the REF6 gene an epigenetic regulator of drought and heat response. 1344 variable markers across 11 drought-linked traits, identifying 28 candidate genes spanning blooming organ development, starch metabolism, and drought regulation.

GWAS applied to nutritional traits and identified 62 significant MTAs explaining up to

28.63% of phenotypic variance for protein, iron, and zinc content under heat and drought stress pointing toward biofortification targets that could simultaneously enhance nutritional quality and stress resilience.

QTL MAPPING AND META-ANALYSIS

The QTL-hotspot on chromosome CaLG04 represents the most thoroughly characterised genomic region for drought tolerance in any grain legume. This single region, spanning 29 cM, contains 12 QTLs controlling multiple root-related and yield traits, accounting for up to 58% of phenotypic variation in root architecture under drought stress. Subsequent refinement using genotyping-by-sequencing (GBS) technology divided the hotspot into QTL-hotspot a (15 genes, 139 Kb) and QTL-hotspot_b (11 genes, 153 Kb).

A comprehensive meta-QTL analysis integrated 1,512 QTLs across diverse studies, identifying 59 meta-QTLs (MQTLs) distributed across all eight chromosomes. Five MQTLs were prioritised as highest-value breeding targets based on high phenotypic variance explained, narrow confidence intervals, and consistency across independent QTL experiments.

Table 1. Selected genomic studies identifying QTLs and MTAs for abiotic stress tolerance in chickpea (*C. arietinum* L.).

Stress	Approach	Key Genomic Finding
Drought	GWAS (429 accessions)	262 MTAs; REF6 as key tolerance gene
Drought	QTL hotspot (GBS)	Hotspot-a (15 genes) & hotspot-b (11 genes) on CaLG04
Drought	SNP genotyping	Ca04468 & Ca07571 for dehydration tolerance
Heat	GWAS (GBS-BLINK)	27 MTAs; GH3.1 auxin-responsive gene identified
Heat	QTL mapping (SNP)	4 major QTLs on CaLG05 & CaLG06
Salinity	GWAS (DARTseq)	28 QTLs on Ca4 & Ca2 chromosomes
Cold	QTL mapping (747 SNPs)	CT Ca-8.1 and CT Ca-3.1 (48% & 34% PVE)
Multiple	Meta-QTL analysis	59 MQTLs across 8 chromosomes; HSPs & auxin response factors

ADVANCED BREEDING TECHNIQUES

Molecular discoveries and bioinformatic insights carry value only insofar as they translate into superior varieties in farmers fields. Advanced breeding technologies provide the translational machinery converting candidate gene lists and QTL

coordinates into stress-tolerant genotypes at unprecedented speed and precision.

Marker-Assisted and Genomic Selection

Marker-assisted selection (MAS) using validated functional markers has already yielded field-level success. The QTL-hotspot for drought tolerance has been introgressed into three elite Indian varieties JG 11, JG 16 and JAKI 9218 via marker-assisted backcrossing, with introgression lines showing measurably improved grain yield under water-limited conditions. Genomic selection (GS) represents a conceptual advance beyond MAS using genome wide SNP data across 3,000 to 60,000 markers to build predictive statistical models for complex quantitative traits. GS is now being scaled using the 90K Axiom array, potentially compressing the chickpea breeding cycle from 8–10 years to 4–5 years by enabling selection before field evaluation.

Speed Breeding

Speed breeding the use of extended photoperiod (up to 22 hours), controlled temperature, and optimised nutrition enables chickpea to complete 4–6 generations per year rather than the conventional. When integrated with genomic selection, this rapid-cycle platform can accumulate favourable alleles at a rate approaching the theoretical limit imposed by genetic recombination rather than calendar time.

Genome Editing

CRISPR-Cas9 and base-editing technologies now permit precise, targeted modification of the chickpea genome without introducing foreign DNA an important regulatory advantage in many producing nations. The application of CRISPR-Cas9 to abiotic stress improvement, noting that gene families identified through transcriptomics (including NAC and ERF transcription factors) constitute prime editing targets. The combination of multi-omics candidate gene identification and genome editing closes the loop from discovery to functional validation with a speed impossible in conventional mutagenesis programmes.

Wild Cicer Relatives as Pre-breeding Resources

The domestication bottleneck stripped many stress-tolerance alleles from cultivated chickpea. Among the eight annual wild relatives, *C. reticulatum* and *C. echinospermum* remain

crossable with the crop and carry verified tolerance to drought, heat, and cold. *C. reticulatum* and *C. pinnatifidum* survive heat stress up to 41.8°C. The salinity resistance of *C. microphyllum* offers a further pre-breeding resource. High-density SNP arrays now enable precise genomic tracking of wild-derived segments through backcross generations, eliminating linkage drag while capturing target alleles.

CONCLUSION

The integration of multi-omics profiling, genome-wide bioinformatic analysis and next-generation breeding technologies has transformed chickpea improvement from an empirical art into a precision science. The availability of the reference genome sequence, the 90K Axiom SNP array and curated databases such as CTDB provides the genomic infrastructure required to link molecular phenotypes with field performance at scale.

Cold stress tolerance is genomically understudied relative to drought and heat, with no validated GWAS-level associations yet established. Functional validation of the hundreds of candidate genes emerging from transcriptomic studies remains a bottleneck the chickpea mutant resource base is thin compared to model legumes. And the translation pipeline from research institute to smallholder farmer in South Asia and sub-Saharan Africa requires sustained investment in extension systems and regulatory frameworks for genome-edited varieties.

The convergence of speed breeding with genomic selection and genome editing creates a realistic pathway to designer cultivars genotypes optimised for specific climatic niches, nutritional profiles, and market requirements. The chickpea has sustained human civilisations for millennia (Figure 1). The molecular, bioinformatic and breeding tools now available give the scientific community genuine grounds for confidence that it will continue to do so even as the climate under which it is grown becomes increasingly hostile.

***Corresponding E-mail:**
vijayatasingh.gpb@gmail.com